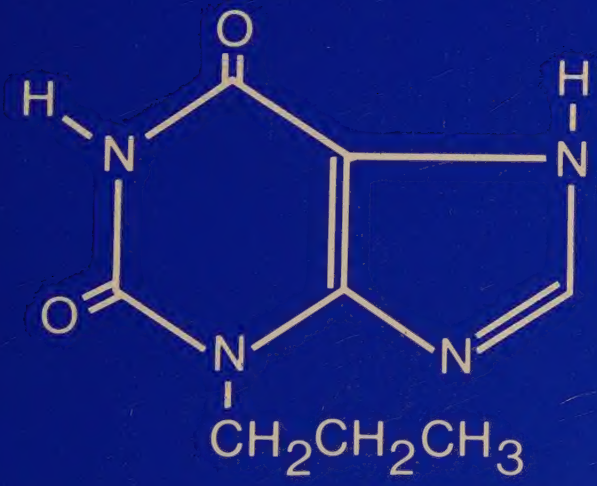
On the Clinical Pharmacology of Enprofylline, a New Antiasthmatic Xanthine

by

ERIK LUNELL



Lund 1983

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AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen
vid Lund Universitet offentligen försvaras
i föreläsningssal F3, Lunds Lasarett
tisdagen den 17 maj 1983 kl 9.15

Lund 1983

Organization
LUND UNIVERSITY
Department of Clinical Pharmacology
University Hospital
S-221 85 Lund
Sweden

Document name
DOCTORAL DISSERTATION
Date of issue
1983-04-26
CODEN:
LUMEDW/(MECF-1001)/1-193/(1983)

Author(s)
Erik Lunell

Sponsoring organization

Title and subtitle

ON THE CLINICAL PHARMACOLOGY OF ENPROFYLLINE, A NEW ANTIASTHMATIC XANTHINE

Abstract

Enprofylline (3-propylxanthine) is about 5 times more potent than theophylline as a bronchodilator and possesses antiallergic and antiinflammatory activities in experimental animals. In contrast to theophylline enprofylline is a poor adenosine antagonist in the systems studied so far. This seems rather beneficial, the most serious side effects of theophylline, namely seizures, are not observed even at high doses of enprofylline in experimental animals.

The pharmacokinetics of enprofylline were studied in 8 healthy subjects after different i.v. and oral doses. Enprofylline in plasma and urine was assayed by HPLC. Enprofylline was well absorbed, negligibly metabolized and excreted unchanged in the urine with a high renal clearance, 200 - 400 ml/min. Volume of distribution was about 0.5 L/kg and protein binding about 50%. Fourteen patients with renal failure showed a reduction of renal drug clearance and protein binding in direct proportion to the degree of renal insufficiency.

Enprofylline produced marked and plasma concentration-dependent bronchodilation in three controlled studies on asthma patients. The plasma concentration interval for bronchodilation, about 1-5 mg/L, suggests the enprofylline/theophylline potency ratio of 5/1 also in man. Enprofylline was well tolerated at broncho-dilating doses. A minor side effect is facial flushing. Headache, nausea and vomiting are probably the dose-limiting adverse effects, apparently plasma concentration-dependent.

Enprofylline differed from theophylline by lacking tremorogenic effects in man. This difference may be attributed to the low ability of enprofylline and potent ability of theophylline to antagonize depressant actions of adenosine in the central nervous system. The results support the notion that adenosine antagonism is neither necessary nor desirable with xanthine antiasthmatics.

Key words
enprofylline, pharmacokinetics, bronchodilation, asthma, tremor, circulatory effects, adenosine receptor antagonism, side effects, theophylline, xanthines.

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISSN and key title

ISBN
91-86058-04-5

Recipient's notes

Number of pages
193

Price

Security classification

Distribution by (name and address) Erik Lunell, M.D.
Department of Clinical Pharmacology, University Hospital, S-22185 Lund, Sweden

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ISBN 91-86058-04-5

Rahms i Lund 1983

Preface

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. O Borgå, K-E Andersson, L-E Edholm, P-O Fagerström, E Lunell, C G A Persson. Pharmacokinetics of enprofylline in healthy subjects after intravenous single dose administration. Submitted for publication.

II. E Lunell, K-E Andersson, O Borgå, P-O Fagerström, G Kjellin, C G A Persson. Pharmacokinetics of enprofylline in healthy subjects after oral single dose administration. In manuscript.

III. E Lunell, O Borgå, R Larsson. Pharmacokinetics of enprofylline in patients with impaired renal function after single dose administration. Submitted for publication.

IV. E Lunell, K-E Andersson, C G A Persson. Tolerance and some circulatory effects of intravenous and oral enprofylline in healthy volunteers. Accepted for publication in Acta Pharmacol Toxicol.

V. E Lunell, N Svedmyr, K-E Andersson, C G A Persson. Effects of enprofylline, a xanthine lacking adenosine receptor antagonism, in patients with chronic obstructive lung disease. Eur J Clin Pharmacol (1982) 22:395-402.

VI. E Lunell, N Svedmyr, K-E Andersson, C G A Persson. A novel bronchodilator xanthine apparently without adenosine receptor antagonism and tremorogenic effect. Eur J Respir Dis. In press.

VII. E Lunell, K-E Andersson, C G A Persson, N Svedmyr. Intravenous enprofylline in asthma patients. Accepted for publication in Eur J Respir Dis.

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BACKGROUND AND INTRODUCTION

Although xanthine-containing beverages in the form of strong coffee were recommended as a remedy of asthma already in the 19th century (Hyde Salter 1859), and the bronchodilating effect of caffeine was known from the turn of the century, it was not until the 1930s that theophylline (1,3-dimethyl-xanthine) was accepted for the medical management of acute asthma (Herrman et al. 1937). In the last decade there has been a worldwide reappraisal of this old drug. Studies defining theophylline's therapeutic range and pharmacokinetic behaviour (Turner-Warwick 1957, Jenne et al. 1972, Jenne et al. 1976, Ogilvie 1978, Hendeles & Weinberger 1980) have provided a rational basis for the therapeutic use of this drug. Today, theophylline is the most widely used bronchodilating drug.

The cumulated research on theophylline has also defined some of its drawbacks. Large intra- and interpatient differences in theophylline clearance due to variability in the rate of hepatic biotransformation (Jenne et al. 1972, Mitenko & Ogilvie 1973) may result in too low or too high plasma levels. Accumulation to high plasma concentrations of theophylline may be associated with serious toxicity (Weinberger et al. 1976, Hendeles et al. 1977). Actually, no clinical symptoms or signs can be relied upon to forewarn the physician of impending toxicity, most feared of all epileptic seizures and cardiac arrhythmias (Zwillich et al. 1975, Jacobs et al. 1976, Rall 1980).

The mechanism of action of theophylline (and other methylxanthines) at the cellular level is not well understood (Rall 1980). When it was discovered that theophylline could inhibit phosphodiesterases, the mode of action of xanthines seemed to be settled (Butcher & Sutherland 1962). However, there is very little inhibition of cyclic AMP hydrolysis at currently used therapeutic plasma concentrations of theophylline (Bergstrand 1980). Several other mechanisms have been proposed

to explain the great diversity of pharmacological effects of theophylline, e.g. interference with intracellular translocation of calcium (c.f. Rall 1980), but again the concentrations needed to exert this effect lie well outside the therapeutic range. A third possibility relates to the ability of theophylline to antagonize the purine nucleoside adenosine.

Adenosine is known to exert powerful effects in a large number of tissues: it inhibits transmitter release, inhibits lipolysis, acts as a vasodilator, depresses cardiac function, inhibits platelet aggregation , etc. Adenosine may thus be a modulator of several biological processes, via an action on specific cell surface receptor sites (Baer & Drummond 1979). Several of the actions of adenosine are antagonized by theophylline at therapeutic concentrations, apparently providing a plausible explanation not only to major extrapulmonary effects such as the CNS-stimulant (Phillis et al. 1979, Daly et al. 1981) and the diuretic effects (Osswald 1975), but also to the antiasthmatic effects (Parker et al. 1978, Fredholm 1980, Snyder 1981, Cushley et al. 1982).

Enprofylline, 3-propylxanthine, is a novel type of antiasthmatic drug, recently developed from careful structure--activity studies on a great number of xanthine derivatives, some of which are shown in figure 1 (Persson et al. 1982). Relaxant effects can obviously be separated from adenosine antagonism, and substitution in the 3-position seems sufficient to produce potent bronchodilators, according to these studies. Enprofylline was well absorbed when given orally to rats and the dose was recovered to more than 90% as unchanged drug in the urine (Persson & Kjellin 1981). In animals, enprofylline is about five times more potent than theophylline as a bronchodilator and an antiallergic and antiinflammatory activity has been demonstrated (Persson & Kjellin 1981, Persson et al. 1981, Andersson Thesis 1982). Enprofylline

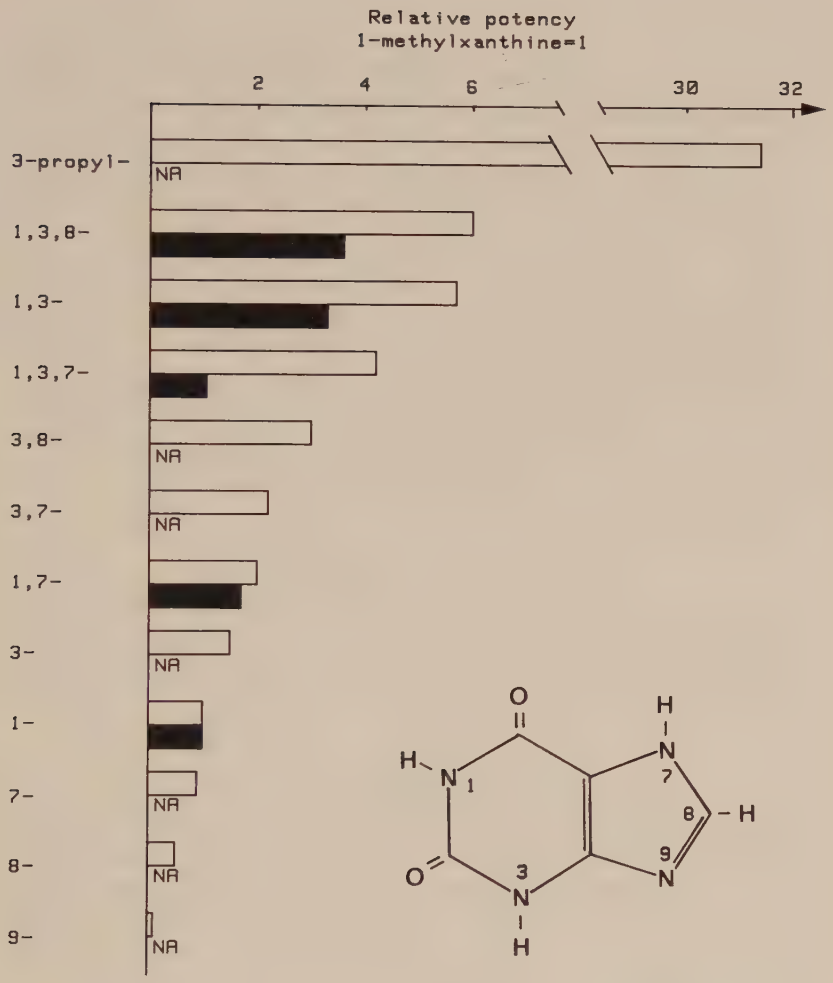

Fig. 1. Some main findings on structure-activity relationships with alkyl-xanthines. Effects on guinea-pig trachea of enprofylline and different methylxanthines are shown (positions for the substitutions are indicated). The open bars show the relaxant potencies. The filled bars show the potencies as antagonists of adenosine-induced relaxation. Comparisons are made with 1-methylxanthine = 1. NA = non-blocking xanthines. Data are from Persson-Karlsson-Erjefält, Life Sciences 1982, with permission of the authors.

in contrast to theophylline, has a negligible adenosine antagonistic activity in the systems studied so far, e.g. smooth muscle, peripheral and central nervous tissues, cardiac tissue, adipose tissue, thrombocytes, etc. (Persson et al. 1982a, b, Fredholm & Persson 1982, Persson et al. 1983). As a possible consequence of this difference at the cellular level the most serious side effect of theophylline, seizures, is not observed in animal experiments even at high doses of enprofylline (Persson & Erjefält 1982). The promising pharmacokinetic and pharmacodynamic profile of enprofylline made this agent an interesting drug candidate. The present thesis summarizes the initial investigations of enprofylline in man carried out to provide a pharmacodynamic and pharmacokinetic basis for further clinical research with this compound.

AIMS OF THE STUDY

The aims of the study were to elucidate the potential clinical value of 3-propylxanthine (PINN enprofylline) by studies of its pharmacokinetic characteristics, its tolerability and bronchodilator potency.

SUBJECTS, PATIENTS AND METHODS

Healthy subjects. A total of 15 (2 female, 13 male) young subjects, adjudged healthy according to physical examination, ECG and routine blood and urine laboratory tests participated in the study. All had creatinine clearance values above 80 ml/min.

Patients with renal insufficiency. Fourteen patients with different degrees of chronic stable renal impairment, the creatinine clearance varying between 3 and 74 ml/min/1.73 m^2, participated. In all studies a history of migraine headaches or epilepsy was an exclusion criterion. This was also obesity and, in females, insufficient contraception.

Asthma patients. Ten ambulant patients (1 female, 9 males) with chronic partially reversible, obstructive lung disease, participated. Reversibility was defined as 15-50% increase in FEV_1 measured 5 min after the inhalation of 5 doses of Bricanyl^R, each dose containing 0.25 mg terbutaline sulphate. Basal FEV_1 values should not differ more than 15% between test days. In one study (VI), two patients had basal FEV_1 values differing more than 15%.

Drug administration. The intravenous (i.v.) infusions of enprofylline were administered during 10 min (20 min in the renal impairment study) using a Tekmar 922 infusion pump. Subjects and patients were non-fasting when receiving the dose. Since the solutions in one of the studies (I) were diluted with saline just prior to injection, using ordinary disposable syringes, the concentrations of enprofylline obtained were measured by HPLC.

In the oral trials the healthy subjects (II, IV) and the patients (VI) were semi-fasting, i.e., a light breakfast was permitted not later than 1 hour before the start of the experiment.

In one study (V) the asthma patients had fasted over-night. In this study a double-dummy technique was employed, using enprofylline in a water drink, together with placebo tablets and Theolair^R tablets (Riker) together with a water drink. In another (VI) rapidly dissolving tablets of theophylline and enprofylline of the same appearance were used.

No xanthine containing food or beverages were permitted for 24 hours preceding all studies with one exception (III).

All oral medication except glucocorticoids was withheld for 56 hours before the studies in asthma patients (V, VI and VII). Spray treatment was withheld at 22.00 h the evening before day 1 of each study and was allowed only between 17.00 and 22.00 on the days of experiments.

In some studies (V, VI and VII) placebo and drugs were given on two or three consecutive days using a double blind, randomized, cross-over design.

In the studies on healthy volunteers the different drug administrations were separated by two weeks.

Sampling and drug analyses. Blood samples (5 ml) were drawn from an antecubital vein via an indwelling catheter. In the i.v. studies we used the arm opposite to that where the infusion was given. Urine was collected in fractions. Collection times varied from 24 hours in the healthy subjects to 60 hours in the patients with severe renal impairment.

Urine volumes were determined by weighing, the weights being translated to volumes assuming a specific gravity of 1.02 g/ml except in the patients with renal impairment where the specific gravity 1.00 g/ml was assumed. Plasma and urine were kept at -20°C until assayed.

Analysis of enprofylline in human plasma and urine. High pressure liquid chromatography (HPLC) was used, employing columns packed with a chemically bonded reversed-phase material of the C_{18}-type. A mobile phase consisting of methanol/water 30/70 (v/v) containing one gram of phosphoric acid per litre was used for plasma analysis. For urine the mobile phase consisted of acetonitrile, tetrahydrofurane and 0.005 M phosphoric acid buffer pH 7.5 (6/2.5/94 v/v/v).

After addition of an internal standard, plasma containing unchanged enprofylline was extracted with a mixture of chloroform-isopropanol. The extraction was performed at alkaline pH to keep enprofylline in the aqueous phase. After precipitation of residual proteins with perchloric acid, the sample was injected onto the chromatographic column. Urine samples were diluted with mobile phase or water and injected onto the chromatographic column.

Quantitation. Quantitation was performed by measuring the peak height ratio between enprofylline and the internal standard and multi-level calibration using a model 3388A Hewlett Packard reporting integrator.

Recovery and precision. The recoveries of enprofylline and its internal standard were complete (100%) in urine and only slightly lower in plasma (94 and 96% respectively).

The data on inter assay precision varied at different concentrations and between plasma and urine but were in no instance worse than 3.5% expressed as coefficient of variation.

A detailed description of the analytical procedures used for determination of unchanged enprofylline in plasma and urine will be given elsewhere (Edholm and Heintz, in preparation). High pressure liquid chromatography was used also in the analysis of theophylline in plasma .

Protein binding. Plasma protein binding was assayed by equilibrium dialysis at room temperature (20°C) as described by Ehrnebo et al. 1971.

Standard laboratory tests. Blood and liver tests, serum-creatinine, and urine examinations were made by the first trial day before drug intake and were repeated after 7-14 days.

Renal function tests. Creatinine clearance was measured using the 24-h urine excretion of endogenous creatinine and a single plasma creatinine determination. Creatinine in plasma and urine was assayed by the Jaffé reaction with an Autoanalyzer (Technicon method M-38). Creatinine clearance values were corrected to 1.73 m^2 body surface area.

Pharmacokinetic calculations. Area under plasma concentration--time curves (AUC) were determined by the trapezoidal rule. AUC from the last plasma concentration, C_n, to infinity was estimated as C_n/β. Beta was estimated as the slope of the apparently linear last portion of the ln C_p *vs* time curves, $t_{½}$ as ln 2/β. Beta and $t_{½}$ were also estimated from the excretion rate *vs* time curves.

Volume of distribution V_{area} or V_β was estimated during the beta elimination phase as CL/β. V_{ss} was calculated as described by Benet and Galeazzi (1979) and Smith et al. (1981):

$$V_{ss} = \frac{\text{Dose} \cdot \text{AUMC}_\infty}{\text{AUC}_\infty} - \frac{\text{Dose} \cdot \text{Infusion time}}{2 \cdot \text{AUC}_\infty}$$

Here $AUMC_\infty$ is the area under the first moment of the plasma curve, i.e., $\int_0^\infty t \cdot c_p \, dt$. This area was measured as the trapezoidal area plus the extrapolated area which in this case is $\frac{C_n}{\beta^2} + \frac{C_n \cdot t_n}{\beta}$.

Distribution volumes based on unbound drug concentrations in plasma, V^u_β and V^u_{ss}, were calculated as V_β/f_u and V_{ss}/f_u, respectively. The unbound fraction in plasma, f_u, is virtually constant at the low concentrations obtained after a dose of 1 mg/kg.

Total plasma clearance, CL, was estimated as Dose/AUC. Renal clearance, CL_r, was estimated by dividing the total recovery, X_n of enprofylline in urine up to a certain time, t_n (usually the last measurable point on the plasma concentration curve) by the corresponding AUC. Thus $CL_r = \frac{X_n}{\int_0^{tn} c_p dt}$. The non-renal clearance, CL_{ren}, was calculated as $CL - CL_r$.

The rate of absorption, expressed as the mean absorption time, MAT (Cutler 1978), was calculated as $MAT = MRT_{oral} - MRT_{i.v.}$ The mean residence time, MRT, obtained in the oral and the i.v. studies on the same subjects were used for the calculations of MAT. MRT is defined (Oppenheimer et al. 1975) as

$$MRT = \frac{\int_0^\infty t \cdot C \, dt}{\int_0^\infty C \, dt}$$

The rate of absorption was also calculated as described by Wagner & Nelson (1963). Employing this method the cumulative amount of drug absorbed $(X_A)_T$ divided by the apparent volume of distribution (V) vs time, plotted for each subject and dose, gives a graphic impression of the absorption kinetics. The formula used was

$$\frac{(X_A)_T}{V} = C_T + K_E \cdot \int_0^T C dt$$

Oral bioavailability (F) in per cent was calculated as

$$F = \frac{AUC^\infty_{oral} \cdot D_{i.v.} \cdot 100}{AUC^\infty_{i.v.} \cdot D_{oral}}$$

where D is the dose. Oral bioavailability (%) was also calculated as the ratio oral/i.v. urine recovery x 100.

The pharmacokinetic parameters after the intravenous and oral administrations were computed with the aid of a pharmacokinetic program, FAKIN, run on an HP-9835 desk computer (Fagerström & Lindmark, unpubl.).

Circulatory parameters. In all studies except one (III) electrocardiogram (ECG) was monitored. Heart rate (HR) and blood pressure (BP) with a manometer were recorded regularly, always by the same investigator.

Spirometry. FEV_1 and vital capacity (VC) were measured using a Bernstein spirometer 60 min before and immediately before drug administration (Basal value) and then regularly every 30 min for 360 min. Each value represents the highest of three consecutive maneuvres. Each day of the experiments was closed by performing spirometry 5 min after the inhalation of 5 doses of Bricanyl^R aerosol, each dose containing 0.25 mg terbutaline sulphate. Spirometry was performed in the patients with asthma (V, VI and VII). FEV_1 = forced expiratory volume in one second.

Tremor recordings. Skeletal muscle tremor was recorded according to Thiringer and Svedmyr (1975). A single plane accelerometer model SPA 1 (Grass) was used. The device with its insulation was fixed to the right hand third finger. Tremor oscillations were recorded (Grass polygraph) as such and via an integrator (Grass) which summed up the deflections for each 10 seconds period. The values were expressed as tremor ratios where each individual patient's basal tremor (obtained after 60 min rest in the start of each experiment) was set to 1.00.

Adverse reactions. In all studies except one (III) the subjects were frequently questioned about side effects according to a protocol. By this means occurrence and duration of headache, nausea and other specified xanthine-like adverse reactions to enprofylline could be quantified.

Statistical methods. The significance of differences between pretreatment and treatment values and between values of different treatments were evaluated by Student's *t*-test. In the study on patients with renal impairment the significance of correlation coefficients obtained by linear regression analysis was assessed by *t*-tests for independent samples. Results corresponding to probabilities less than 0.05 in a two tailed test were considered statistically significant. The results are given as mean ± SD or as mean ± SEM.

REVIEW OF THE RESULTS

I. Pharmacokinetics of enprofylline in healthy subjects after intravenous single dose administration

Eight healthy subjects received single doses of enprofylline 0.5, 1.0 and 1.5 mg/kg, administered as an infusion over 10 minutes. Plasma levels of unchanged enprofylline were followed for 3-8 hours. In general the plasma fall-off curves were log--linear from one hour on, in many cases from 30 minutes after

drug administration. Volumes of distribution (V_{area} and V_{ss}) averaged 0.5 L/kg.

Renal clearance (CL_{ren}) values were high, 200-400 ml/min, and most of the total clearance (CL) was accounted for by renal clearance. The high renal clearance indicated that the major elimination route for enprofylline is by active tubular secretion. Plasma half-life varied from 1.2 to 1.9 hours. When a drug is eliminated only in the urine, the total clearance is its renal clearance, and the elimination rate is the urinary excretion rate. There was a good agreement between half-lives calculated from the slope of excretion rate *vs.* time curves and those calculated from plasma levels, the average half-lives from plasma data being only slightly shorter.

Areas under the plasma concentration *vs.* time curves (AUC) up to 2 hours, normalized for differences in dose, were elevated by about 8% at the highest dose level compared to the middle dose level ($p < 0.01$). Also total clearance values were decreased by about 9% for the highest dose compared to the middle dose ($p < 0.05$). This provided evidence for a slight dose-dependency in CL and CL_{ren} that might be more pronounced at higher doses. The basic pharmacokinetic parameters obtained in the study are shown in Table 1.

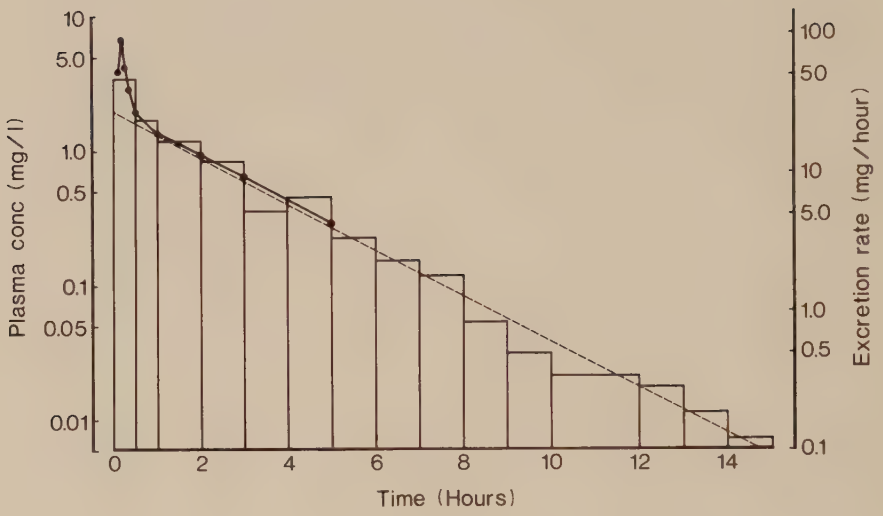

Fig. 2. Plasma concentrations (●—●) and excretion rates in urine (bars) of enprofylline in one subject (No. 6) after 10 min i.v. infusion of 1.5 mg/kg. Note the two different scales on the ordinate axis. The mid time--points of the excretion rate bars were fitted to a straight line (broken) with linear regression analysis starting from the second fraction (30-60 min).
S.I. conversion: micromole/L = mg/L · 5.1

Table 1. Kinetic parameters obtained in 8 subjects after 10 min i.v. infusion of enprofylline (mean ± SD).

Dose (mg/kg)	0.5	1.0	1.5	Mean ± SD of 3 dose-levels
$T_{½}$ plasma (hours)	1.59	1.63	1.64	1.62 ± 0.24
$T_{½}$ urine (hours)	1.71	1.66	1.70	1.69 ± 0.23
V_{area} (L/kg)	0.58	0.59	0.54	0.57 ± 0.08
V_{ss} (L/kg)	0.52	0.53	0.49	0.51 ± 0.06
CL_{tot} (L/hr/kg)	0.26	0.25	0.23	0.25 ± 0.04
CL_{ren} (mL/min)	300 (n = 7)	277 (n = 6)	262 (n = 7)	279 ± 44
Urine recovery (%)	89.9 (n = 7)	86.5 (n = 6)	89.8 (n = 7)	89.1 ± 4.2

II. Pharmacokinetics of enprofylline in healthy subjects after oral single dose administration

Enprofylline, given orally to healthy subjects in single doses of 2, 4 and 6 mg/kg produced plasma concentrations in the range 1-6 mg/L, the two lower doses in the assumed "therapeutic interval", 1-4 mg/L, according to previous animal studies. An initial rapid increase in plasma drug concentration was followed by double peaks in 5 of the 6 subjects at the higher dose levels. The individual enprofylline peak levels showed some variation, but increased with increasing dose. The mean absorption time (MAT), which is independent of the shape of the plasma concentration curve or the occurrence of multiple peaks, increased with increasing dose from 0.58 hours after 2 mg/kg to 1.60 hours after 6 mg/kg enprofylline. MAT is the mean time which a drug molecule spends at the input side before being absorbed (Cutler 1978).

The rate of absorption was also calculated as described by Wagner & Nelson (1963). Employing this method, the cumulative amount of drug absorbed, divided by the apparent volume of distribution (V) versus time could be plotted for each subject and dose, to give a graphic impression of the absorption kinetics, that allows the simultaneous comparison of both the rate and the extent of enprofylline absorption. An example is shown in Fig. 3 . The absorption was apparently terminated 1-2 hours after the 2 mg/kg dose but still continuing 6 hours after the higher doses in half of the subjects.

The areas under the plasma concentration-time curves (AUC^{∞}) showed a nonlinear increase with increasing dose, as did bioavailability calculated as $AUC^{\infty}_{or}/AUC^{\infty}_{i.v.}$. The data indicated capacity limited elimination kinetics rather

than absorption increasing with dose, since bioavailability calculated from plasma data exceeded 100% at the highest dose level. The dose dependency was also apparent from some of the Wagner-Nelson plots, where the plateau values increased disproportionally with increasing dose. Recovery of unchanged drug in urine, collected for 24 hours, averaged about 90%, indicating high oral absorption and negligible metabolism.

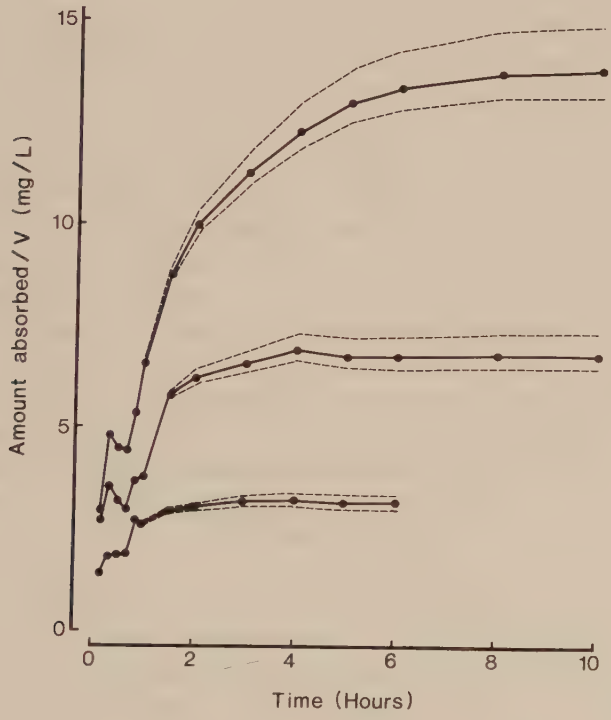

Fig. 3. Rate of drug absorption illustrated by Wagner-Nelson plots of plasma concentration data obtained in subject No. 3 after oral administrations of enprofylline. The elimination rate constant (β) used for the calculations was the mean value from experiments with three i.v. doses in paper I. Dispersion denotes this subject's extremes in β value.

Multiple peaks in the plasma drug concentrations and a prolongation of the MAT at the higher dose levels, indicated a delayed gastric emptying, as did the Wagner-Nelson absorption rate curves. The short half-life of enprofylline, 1.2-1.9 hours, will, however, necessitate the use of controlled-release preparations, from which the main part of absorption will take place in the intestine. The absorption rate of enprofylline is obviously high after the passage through the pylorus.

III. Pharmacokinetics of enprofylline in patients with impaired renal function after intravenous single dose administration

Plasma and urine concentrations of unchanged drug after a single i.v. dose of 1 mg/kg enprofylline were followed in 7 patients with severe renal impairment, creatinine clearance 3-18 ml/min (patient group I), 7 patients with moderate to slight renal impairment, creatinine clearance 31-74 ml/min (patient group II) and 7 healthy subjects.

The mean plasma concentrations of enprofylline were higher immediately after termination of the infusion in group I compared to group II and to the healthy subjects. The mean plasma concentrations in group I 4 hours after dose were about 6 times as high, and in group II 3 times as high as in the healthy subjects.

Enprofylline renal clearance varied from 2.1 to 357 ml/min and was strongly related to creatinine clearance ($r = 0.94$). Also non-renal clearance was lower in patients with severe renal insufficiency than in healthy subjects, significantly related to creatinine clearance ($r = 0.74$, $p < 0.001$). However, there was also a decrease in non-renal clearance with increasing age ($r = 0.67$, $p < 0.001$). Plasma half-lives were prolonged as the creatinine clearance was

reduced. Marked prolongation of the plasma half-life did not occur until renal function was severely compromised (Fig. 4). In group II patients the plasma half-life of enprofylline ranged between 2.7-7.2 hours and in group I between 8.6-26.6 hours, compared to 1.5-2.6 hours in the healthy subjects.

Volumes of distribution (V_{β} and V_{ss}) were correlated to creatinine clearance, lower values being obtained in patients with impaired renal function. Calculations of the volume terms using unbound drug levels enhanced this tendency. The decreases in V_{ss} together with the decreases in CL_{ren} and $CL_{non-ren}$ may explain the high plasma concentrations of enprofylline already after termination of the infusion in the patients with renal insufficiency.

The unbound fraction of enprofylline in plasma increased from 55% in the healthy subjects to 66% in the group of patients with the highest degree of renal impairment. Recalculated values of the plasma protein binding to a "normal value" of 45 g/L of serum albumin still showed an increasing unbound fraction with decreasing creatinine clearance ($r = -0.67$, $p < 0.001$) indicating that this phenomenon could not be explained by lower albumin in patients with renal impairment, binding inhibitors present in plasma being the most probable explanation. The changes in pharmacokinetics of enprofylline may give an altered pharmacological response in renal failure.

Very little is known about the metabolism of enprofylline. In the group I patients non-renal clearance was of the same order of magnitude as the renal clearance and in group II the non-renal clearance still accounted for about 1/4 of the total clearance. However, in absolute figures, the non-renal clearance decreased with decreasing

renal function, which would rule out any induction of metabolism of enprofylline in patients with impaired renal function.

Plasma levels above 1 mg/L throughout the dosage interval would be obtained using the following preliminary doses and dose intervals of enprofylline intravenously.

0.75 mg/kg every 12 h in patients with creatinine clearance < 20 ml/min or serum creatinine > 300 µmole/L;

1.0 mg/kg every 6 h in patients with creatinine clearance 30-50 ml/min or serum creatinine > 150 µmole/L;

1.0 mg/kg every 4 h in patients with creatinine clearance 50-75 ml/min or serum creatinine > 115 µmole/L.

However, experience of repeated dosing in renal impairment is needed to give definite dose recommendations according to the degree of renal insufficiency.

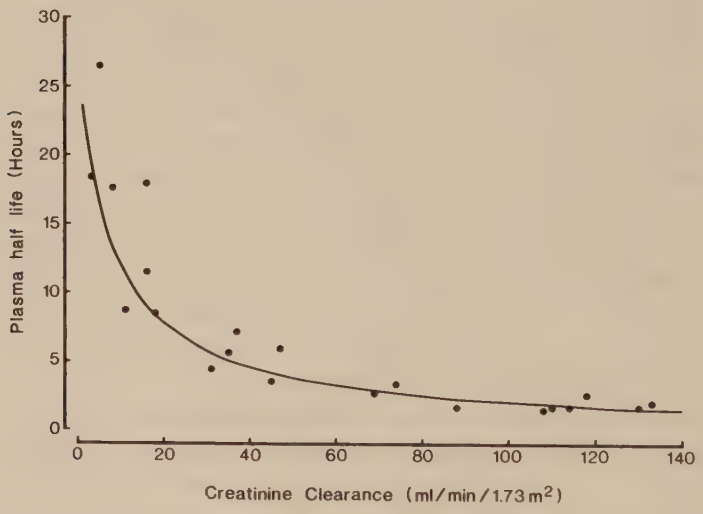

Fig. 4. The enprofylline half-life, following a single i.v. administration of a 1 mg/kg dose, in 7 healthy subjects and 14 patients with varying degrees of renal impairment.

IV. Tolerance and Some Circularory effects of intravenous and oral enprofylline in healthy volunteers

Enprofylline given intravenously (i.v.) to recumbent healthy volunteers in the doses 0.5, 1.0 and 1.5 mg/kg and orally in the doses 2, 4 and 6 mg/kg produced mean plasma levels in the range 1.6-4.4 mg/L after the i.v. and 1.9-5.5 mg/L after the oral administration, i.e. in the assumed "therapeutic interval".

In the supine position a slight but significant increase (< 10 beats/min) in heart rate was seen at peak plasma levels after each of the highest i.v. and oral doses. Positive chronotropic and vasodilator actions could be predicted from experiments on animals (Persson et al. 1983). In orthostatic tests heart rate and blood pressure (BP) were measured immediately after rising to standing position and after 2 and 4 min of quiet standing. The normal cardiovascular adaptation to standing consists of an increase in heart rate, a decrease in systolic blood pressure and a rise in diastolic pressure (Drischel et al. 1963; de Marées 1976). The normal pattern of orthostatic response was retained also after enprofylline, although slightly augmented at the time points of the maximum plasma concentrations. Enprofylline thus increased the orthostatic heart rate response after the highest i.v. dose and after 4 and 6 mg/kg orally (Fig. 5). A slight decrease in systolic BP was observed in the orthostatic tests performed immediately after the infusion of 1.5 mg/kg and 60-120 min after oral ingestion of 4 and 6 mg/kg. The only effect on diastolic BP attributable to the drug was a short-lasting decrease immediately after infusion of 1.5 mg/kg, possibly reflecting that the vasodilator properties of enprofylline overcame the normal reflex induced vasocon-

striction. The heart rate and BP responses were of the same magnitude after 2 and 4 min standing.

Adverse reactions (Table 2) occurred most frequently after the two highest i.v. and oral doses and were mild and transient. Nausea and headache were noted in 5 of the 24 i.v. and in 9 of the 17 oral experiments. In cats and dogs enprofylline was slightly more potent than theophylline to produce nausea, and it was suggested that the nauseatic effects are unrelated to adenosine antagonism (Persson & Erjefält 1982). The headache was of a pulsating type, making the vasodilator action of enprofylline a probable contributing factor. Also this action may be unrelated to adenosine antagonism. Both nausea and headache may be xanthine-induced effects that are pronounced in drug-naive subjects and may subside by continuous treatment (cf. Rall 1980). A facial flush lasting for less than 3-4 min was noted by two subjects immediately after infusion of the two larger doses of enprofylline. The flush was probably also a sign of vasodilation.

In conclusion, the circulatory effects of enprofylline were small and the adverse reactions mild. They could not be considered a hindrance for further clinical evaluation of the drug.

Table 2. Number of adverse reactions after i.v. and oral enprofylline in healthy volunteers

	Dose mg/kg	Nausea	Headache	Flush	Other
i.v.	0.5	-	-	-	-
	1.0	1	2	2	-
	1.5	3*	-	2	-
oral	2	1	1	-	-
	4	4	2	1	-
	6	4	2	-	1 (epigastric pain)

*2 subjects vomited

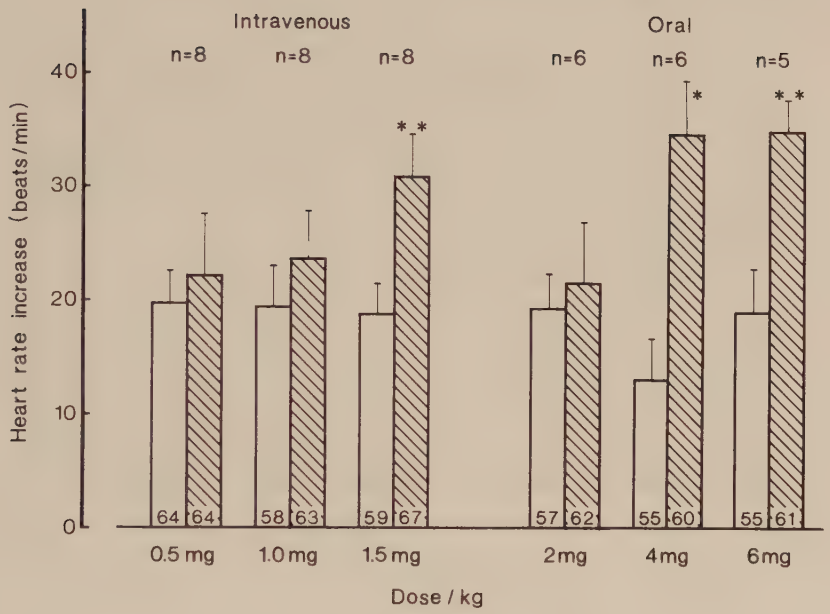

Fig. 5 Changes in pulse rate at different dose levels in orthostatic tests performed before drug administration, immediately after termination of drug infusions and 60 minutes after oral intake of enprofylline in healthy subjects. Values after 2 min in upright position are given (mean ± SEM). Open bars=before drug. Hatched bars=after drug. **= $p < 0.01$; *= $p < 0.05$. At the bottom of each bar is given the mean supine heart rate before the orthostatic test.

V. Effects of enprofylline, a xanthine lacking adenosine receptor antagonism, in patients with chronic obstructive lung disease

This study was preceded by a pilot experiment in two asthmatic patients to investigate whether or not enprofylline had bronchodilating effect in asthma. The first patient to receive enprofylline was a man of 57 years with a duration of illness of 13 years. The result of the spirometry is shown in Fig. 6

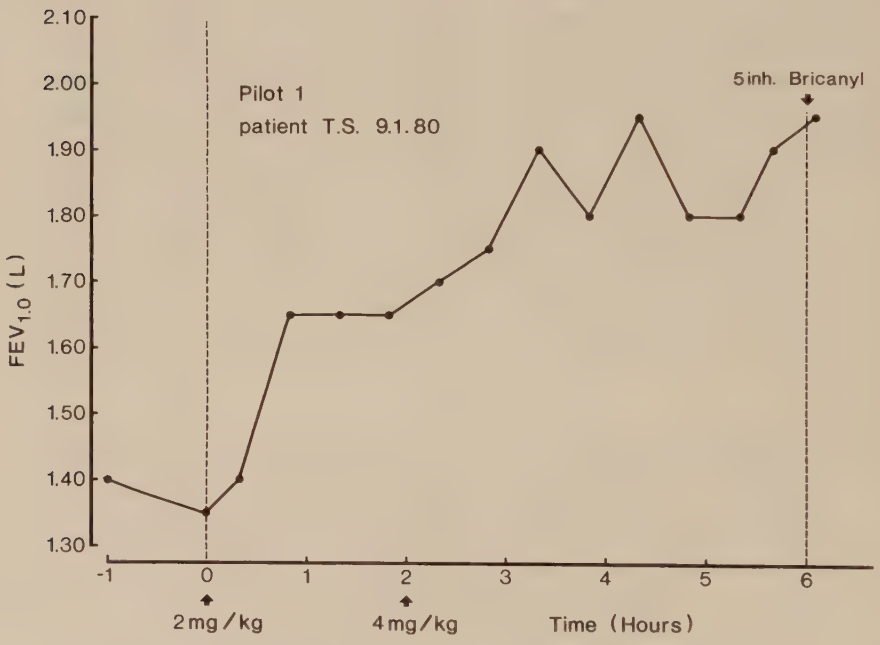

Fig. 6. Changes in forced expiratory volume in the first second (FEV_1) in the first patient receiving enprofylline. The dosage was 2 mg/kg initially, followed 2 hours later by 4 mg/kg, given orally in a water solution. The day was closed by the inhalation of 5 doses of Bricanyl^R aerosol, each containing 0.25 mg terbutaline sulphate.

Eight adults with chronic, partly reversible, obstructive lung disease then participated in a double-blind, cross-over study to compare bronchodilation obtained with 1 day's oral treatment of each of enprofylline, theophylline and placebo. The placebo treatment separated the two active treatments. The dosage of enprofylline 2 mg/kg initially, followed 2 hours later by 4 mg/kg, produced maximum enprofylline levels of 3.0 mg/L and 8.5 mg/L, respectively. The assumed corresponding doses of theophylline 4 mg/kg initially and 6 mg/kg 2 hours later, produced maximum plasma levels of 7.2 mg/L and 18.5 mg/L, respectively (mean values).

The maximum bronchodilation was two-fold greater on enprofylline (17% increase in FEV_1 over baseline) than on theophylline (9%) after the initial doses, but similar on both treatments (32 vs. 31%) after the second dose. Both doses of enprofylline produced higher heart rates than corresponding doses of theophylline. The patients were unaware of any cardioaccelerating effects of enprofylline. Tremor of the hands was noticable after theophylline but not after enprofylline.

Side effects on the initial lower doses were flushing in one patient after enprofylline and palpitations in one patient after theophylline. Three patients reported headache and two reported brief nausea and gastric discomfort after the high dose enprofylline. Different dosage forms were used with enprofylline solution and theophylline tablets (double-dummy technique) and the results suggest that the doses given may not be equipotent for bronchodilation.

It was concluded that enprofylline is a potent bronchodilator in man.

VI. A novel bronchodilator xanthine apparently without adenosine receptor antagonism and tremorogenic effect

Eight patients with asthma received single oral doses of theophylline (9.5 mg/kg) and enprofylline (3.5 mg/kg) in a double-blind, randomized, crossover trial on two consecutive days. Tremor of hands, first second expiratory volume (FEV_1), vital capacity, heart rate and blood pressures were followed for 6 hours. Plasma concentrations of both drugs were monitored.

The two drugs produced significant bronchodilation of similar magnitude. Enprofylline did not change the tremor of hands from the basal value, whereas theophylline produced a significant increase in tremor compared to the basal values and to the enprofylline trial values (Fig. 7).

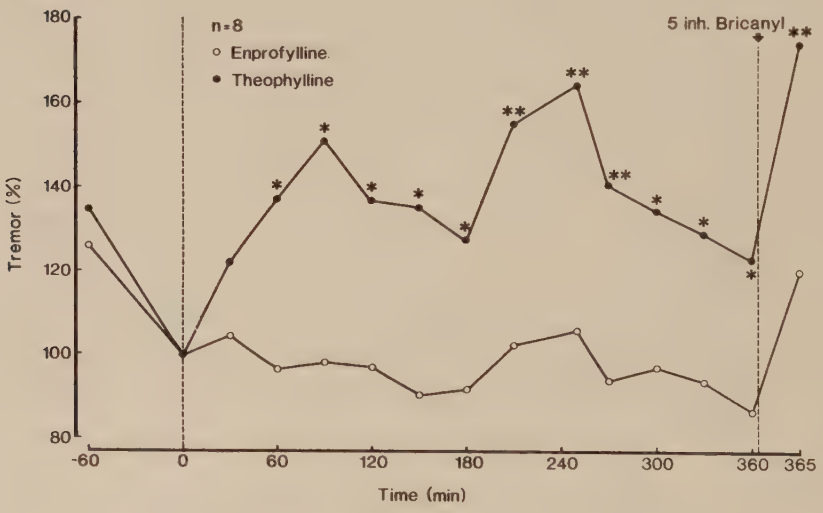

Fig. 7. Average tremor of hands after single "equipotent" oral doses of theophylline and enprofylline. Each individual patient's basal tremor was set to 100%. Asterisks denote significant difference between theophylline and enprofylline at different times after intake. * = $p < 0.05$; ** = $p < 0.01$.

The onset and intensity of the tremor were correlated to the plasma levels of theophylline. The mean relative amount of tremor (expressed in per cent of each individual patient's basal tremor) was 151 and 164%at 90 and 250 minutes after intake of theophylline. The corresponding values recorded after intake of enprofylline were 98 and 106%, respectively. Five inhalations of Bricanyl aerosol, each dose containing 0.25 mg terbutaline sulphate, at the end of the experiments produced an additional increase in tremor. A light meal after 180 min increased the tremor somewhat and seemed to be the only additional factor that influenced the tremor. The tremor can normally be felt by the patient at a level of 160% of basal tremor (cf. Thiringer & Svedmyr 1975). It was suggested that tremor by methylxanthines is a sign of CNS-stimulant effects and that the difference in tremorogenic action between enprofylline and theophylline reflects the lack of potency of enprofylline to antagonize adenosine receptors in the CNS.

VII. Intravenous enprofylline in asthma patients

Enprofylline, 1 mg/kg was infused i.v. during 10 minutes at 60-minutes intervals in an acute multiple-dose (3 doses) study on eight patients with asthma. The design was a double blind crossover spirometry comparison with placebo (0.9% NaCl).

Fifty minutes after each infusion, when the distribution phases, according to the shape of the plasma concentration curves, apparently were terminated the mean plasma concentrations were 1.37, 2.54 and 4.25 mg/L, respectively. At these instances the mean increases in FEV_1 were 14.1, 24.7 and 28.3%. When the changes in FEV_1 during the placebo experiment were subtracted the mean FEV_1 increases were 9.8, 18.8 and 28.8%, respectively. The graded increments

in FEV_1 corresponded well to the cumulated drug concentrations in plasma. However, it cannot be excluded that part of the effects after the second and third doses were due to a slowly developing effect of the previous doses.

An additional increase in FEV_1 of 14.1% was produced by inhalation of 1.25 mg terbutaline sulphate at the end of each trial day. The data suggest that enprofylline produces significant and concentration-dependent bronchodilation at least at plasma levels in the range 1-4 mg/L.

No change in tremor of hand and no subjective side effects were recorded. Heart rate was increased by maximally 13 beats/min by enprofylline (mean value). In conclusion, enprofylline given i.v. is well tolerated and produces marked and dose-dependent bronchodilation.

GENERAL DISCUSSION

In modern theophylline treatment of asthma, one has been facing two main problems of obtaining a safe and optimal therapy. Firstly, a largely unpredictable elimination rate (Jenne et al. 1976, Mitenko & Ogilvie 1973, Ginchansky & Weinberger 1977) and, secondly, a narrow therapeutic range with serious toxicity associated with excessive plasma concentrations (Zwillich et al. 1975, Jacobs et al. 1976, Hendeles et al. 1977)

Pharmacokinetics. The elimination half-life of theophylline in healthy adults varies from less than 3 to over 15 hours, primarily due to a large variability in the rate of hepatic transformation, which can also change with age, smoking habits, concomitant drugs, aberrations in diet, and certain diseases (Riegelman et al. 1980, Hendeles & Weinberger 1980). Non-linear, or dose-dependent, elimination caused by capacity limited metabolism has also been demonstrated

with therapeutic doses of theophylline (Tang-Liu 1982). The main metabolite of theophylline, 3-methylxanthine (Jenne et al. 1976), that possesses 1/5 - 1/3 of theophylline's bronchodilating potency (Persson & Andersson 1977), is eliminated unchanged (Jenne et al. 1976) with high renal clearance, 170-233 mL/min (Tang-Liu 1982).

The pharmacokinetics of enprofylline were studied in healthy subjects (I and II) and in patients with renal insufficiency (III). The extent of oral absorption of enprofylline was 90-100% of the given dose. A high variability in the rate of absorption of different doses of enprofylline was probably related to intermittent and delayed passage from the stomach into the small bowel, which would be the optimal site of absorption of enprofylline. Such delayed gastric emptying was less obvious at the lowest dose-level (II).

The distribution phase after i.v. administration appeared from plasma data to be complete within 30-60 min. The almost perfectly linear log excretion *vs*. time plots did not indicate more than one elimination phase, which would suggest the absence of "deep compartments" of any significant size (I). Volume of distribution averaged 0.5 L/kg body weight, which is similar to that of theophylline (Riegelman et al. 1980). Since protein binding in plasma was almost identical to that of theophylline in healthy subjects, i.e. about 47% (Tegnér et al., unpublished and III), the over-all tissue distribution of the two drugs appears to be similar.

The unbound fraction of enprofylline increased with decreased renal function (III). The increase was approximately 10% in the group of patients with the most severely compromized renal function. Values of the unbound fraction, recalculated for decreased albumin levels, still increased with decreasing creatinine clearance. Binding inhibitors

present in uremic plasma is the most probable explanation to this phenomenon (cf. Sjöholm et al. 1976).

Volumes of distribution (V_β and V_{ss}) decreased with renal function impairment, and recalculations using unbound drug levels enhanced this tendency. This is contrary to what could be expected since the apparent volume of distribution generally tends to increase when plasma binding is decreased (Gibaldi 1978, Jusko & Chiang 1982). However, our healthy subjects were considerably younger than our patients, and there may exist an age dependency for the volume of distribution of enprofylline, similar to that demonstrated for theophylline (Anthal et al. 1981). A study of the pharmacokinetics of enprofylline with special reference to the influence of age therefore seems warranted.

Half-lives in the healthy subjects calculated from urinary excretion rates correlated well with plasma half--lives (1.2-1.9 hours), being only slightly longer (I and III). The renal clearance of enprofylline, 200-400 ml/min, indicated active tubular secretion as the major elimination route, since only about 50-80 ml/min of the renal clearance may be accounted for by glomerular filtration. A reduction in renal clearance of enprofylline is also seen in healthy subjects after pretreatment with 1 g probenecid according to preliminary data (Borgå et al., unpublished). The present study was not designed to evaluate the mechanisms of renal elimination of enprofylline, e.g. whether tubular absorption occurs, which remains to be studied. There was a large variation in renal clearance of enprofylline in healthy subjects compared to the variability in creatinine clearance, which makes it desirable to study the relationship between the two parameters in a larger population. The pharmacokinetics in asthma patients (V-VII) did not appear to be different from those observed in healthy volunteers.

In patients with impaired renal function (III) plasma half-lives were prolonged and total and renal clearances reduced in direct proportion to the creatinine clearance. In the patients with severe renal impairment non-renal clearance was of the same order of magnitude as the renal clearance (about 15 ml/min) and in the patients with moderate to slight renal impairment the average non-renal clearance still accounted for 1/4 of the total clearance. However, in absolute figures the non-renal clearance decreased with decreasing renal function. The reason for this may, at least to some extent, be differences in age, since there also was a significant inverse relationship between age and non-renal clearance of enprofylline.

Both increased and decreased rate of metabolism have been associated with uremia (Maddocks et al. 1975, Larsson et al. 1982). The metabolic pathways of enprofylline have so far not been identified. The incomplete recovery in urine (82-89%) of healthy subjects during a sampling time exceeding 10 half-lives suggests the existence of other elimination routes. Theoretically enprofylline could, in similarity with theophylline, be metabolized via oxidation at the C_8-position, yielding 3-propyluric acid. This metabolite would then possibly be detectable in the plasma from patients with uremia given enprofylline. Any speculation on the reason for the observed decrease in non-renal clearance of enprofylline in patients with impaired renal function is, however, impossible , until such investigations have been conducted.

Pharmacodynamics. There is growing evidence suggesting that extracellular adenosine, by acting at external cell membrane receptors, causes alterations in the response of the target cell in many tissues (Fredholm 1980, Burnstock 1981, Baer & Drummond 1979). Adenosine may act as a local regulator of cell function already under basal physiologi-

cal conditions in some tissues. In other tissues adenosine may be important only when the concentrations are raised, for example by ischemia, by sympathetic overstimulation, or by drugs, e.g. β-adrenoceptor stimulants (Berne et al. 1979, Schrader 1981).

Based primarily on biochemical studies adenosine receptors have been divided into two subclasses, A_1 (or Ri) and A_2 (or Ra), by van Calker et al. (1979). Stimulation of the A_1-receptor has been associated with a decrease in cyclic AMP, e.g. in rat adipose tissue, and stimulation of the A_2-receptor with an increase in cyclic AMP, e.g. in rat hippocampus. Theophylline at low concentrations antagonizes actions of endogenous adenosine at either of these receptor types (Londos & Wolff 1977). In different systems representing the two putative adenosine-receptor subclasses, almost identical rank of order of antagonist activity was found for a number of xanthines (Fredholm & Persson 1982). Irrespective of the adenosine receptor type enprofylline seems to be a poor antagonist (Table 3).

Table 3. Adenosine-receptor-mediated actions

	Antagonized by	
	Theophylline	Enprofylline
Adenosine A_1-receptor functions		
Nervous depression (CNS, periphery)	yes	no
Inhibition of FFA-release	yes	no
Cardiac depressant actions	yes	no
Adenosine A_2-receptor functions		
Smooth muscle relaxation	yes	no
Thrombocyte antiaggregatory effect	yes	no

This table is based on published (e.g. Persson 1982) and unpublished material (e.g. Vinge et al. 1983).

Cardiovascular system. Adenosine increases coronary blood flow, exerts negative inotropic and chronotropic effects in the heart and reduces cardiac oxygen demand (Berne et al. 1979, Schrader 1981). Furthermore, adenosine inhibits the effects of β-receptor stimulation in the heart (cf. Schrader 1981). Since adenosine production is enhanced by catecholamines, it may act as a feed-back inhibitor of the catecholamine effects. Hypoxia and β-receptor stimulation increase the formation of adenosine in the heart (Berne 1979, Schrader 1981), and it has been proposed that adenosine serves as a protective autacoid in the heart (Schrader 1981, Dobson 1982).

Stress and reduced arterial oxygen tension (PaO_2) associated with severe respiratory failure should lead to increased tissue levels of adenosine and it is known that cardiac disturbances are associated with this condition (Senior et al. 1979). Theophylline is known to lower the threshold for arrhythmias under these circumstances (Horowits et al. 1975) and it is conceivable that an adenosine receptor non-blocking xanthine, such as enprofylline, is less cardiotoxic under conditions of increased adenosine influence on the heart (Persson et al. 1983). These aspects have not been specifically addressed in this study, but some cardiovascular findings were obtained. Cardiac arrhythmias or other disturbances attributable to the drug in the electrocardiograms (ECG) were not recorded in any study where continuous ECG monitoring was used (IV-VII), despite high plasma concentrations of enprofylline obtained at some occasions (IV-V).

When given to healthy subjects i.v. at three and orally at three different doses producing plasma concentrations well within and slightly above the tentative bronchodilating range, enprofylline produced small circulatory effects, i.e., a moderate increase in heart rate only after the highest doses in the supine position (IV). In asthma patients, how-

ever, a significant increase in heart rate, apparently concentration-dependent (VII), and a slight decrease in blood pressure occurred in semi-recumbent position (V-VII). Positive chronotropic and vasodilator effects could be predicted from experiments on animals (Persson et al. 1983). The highest doses of enprofylline given to healthy subjects (IV) augmented the normal response to orthostatic tests in healthy subjects, i.e. a heart rate increase and a fall in systolic blood pressure. This possibly reflected that the vasodilator properties of enprofylline overcame the normal reflex vasoconstriction response. The circulatory effects were of short duration and related to plasma peak levels of the drug. These results should be compared with the dose-related increases in heart rate and systolic blood pressure observed with theophylline (e.g. Vestal et al. 1982). The prominent subjective symptom of vasodilation was a shortlasting feeling of facial flushing, that was related to the plasma peak levels of enprofylline after the higher doses given to healthy subjects (IV).

Lung. Different investigators have suggested a role for adenosine in asthma. Some characteristics of this purine nucleoside apparently make it look like an asthma mediator. Lung tissue levels of adenosine are increased by e.g. stress and hypoxia (Mentzer et al. 1975). In vitro adenosine may produce slight bronchoconstriction and it may potentiate allergen induced mediator release (e.g. Peters et al. 1982). Given as inhalation to asthma patients it produces significant bronchoconstriction, but normals do not respond (Cushley et al. 1983). Furthermore, at clinically relevant concentrations theophylline effectively inhibits the actions of adenosine, by specific antagonism at cell surface receptors. The proposals that the antiasthmatic effects of theophylline reflect adenosine antagonism

(Marquardt et al. 1978, Fredholm et al. 1979, Snyder 1981, Cushley et al. 1983) may thus look quite attractive. One way to examine the relevance of these proposals clinically would be to study whether or not bronchodilation can be induced in asthma patients by a xanthine derivative that has a negligible ability to antagonize adenosine.

Enprofylline was shown to be 5 times as potent a bronchodilator as theophylline in animals and in isolated human bronchi (Persson et al. 1981, Persson & Kjellin 1981) but, in contrast to theophylline, enprofylline does not seem to antagonize adenosine (Persson et al. 1981).

The bronchodilator effects of enprofylline were assessed in three double blind, cross-over studies in comparison with theophylline and with placebo. The first, dose-finding, study of enprofylline on asthma patients (V) also included a comparison with theophylline. The design included a moderate oral dose given of each drug, followed by a "high" dose of the drug two hours later. This study was designed to reveal any bronchodilating effect of the drug in man. The lowest dose chosen should be effective if there was a correlation between preclinical and clinical bronchodilator potency of enprofylline. The upper dose should be within the region that had been determined to be safe in the preceding studies on healthy subjects (IV). The results suggest that the doses given may not be equipotent for bronchodilation. Bronchodilation was two-fold greater on enprofylline than on theophylline after the initial doses, suggesting that the enprofylline doses used were too high compared to the theophylline doses. No difference in the maximum effects was observed after the second dose, suggesting that near maximum effects obtainable with xanthines in these patients were produced by both drugs. However, enprofylline proved to be a potent bronchodilator also in man.

In the second study on asthma patients (VI) single doses of enprofylline and theophylline were selected that would produce about equal bronchodilation. This study supported the enprofylline/theophylline potency ratio of about 5/1 for bronchodilation obtained in preclinical experiments.

In the third study (VII) we used an acute multiple-dose design with i.v. enprofylline given in repeated infusions every hour for three successive doses. We were thus able to assess the graded increments in FEV_1 at pseudo-distribution equilibrium and the plasma levels needed to produce the clinical antiasthmatic effect. The data obtained in this dose-response study suggest that enprofylline produces concentration-dependent bronchodilatation at least in the interval 1-4 mg/L plasma. This concentration-response relationship has to be confirmed in a larger number of patients, using designs that allow true steady-state plasma levels to be obtained and where carry-over effects of preceding doses are avoided.

The repeated findings (V-VII) of bronchodilating effects of enprofylline, at plasma concentrations where no adenosine antagonism is expected, are evidence against the view that antiasthmatic effects of xanthines reflect adenosine receptor antagonism. The bronchodilator effects of enprofylline have been confirmed in larger studies by other investigators (Laursen et al. 1983).

Tremor of hands. In the central nervous system (CNS) adenosine inhibits the firing of most neurons (Phillis et al. 1979). Inhibitory actions of adenosine may be mediated largely by the ability to block the release of various neurotransmitters (e.g. Sawynok & Jhamandas 1976). Cumulating experimental evidence suggests that methyl-

xanthines exert also their behavioural stimulant actions through blockade of adenosine receptors (Maitre et al. 1974, Phillis et al. 1979, Dunwiddie et al. 1981).

The stimulant effects of methylxanthines on the CNS have been known for a long time. Effects of excess theophylline on the cerebral cortex start with anxiety, irritability, restlessness, tremor and insomnia and may progress to agitation and convulsions. Life-threatening epileptic seizures are observed mainly when plasma concentrations exceed 30 mg/L. No clinical signs can apparently be relied upon to forewarn the physician of impending serious toxicity (Zwillich et al. 1975, Hendeles & Weinberger 1977).

In animal studies, enprofylline was devoid of theophylline-like CNS-stimulant behavioural effects (Persson et al. 1981). Lethal doses (twice as large as those of theophylline) were without seizure activity. Death was preceeded by sedation and inhibition of activity, which was in contrast to the lethal effects of theophylline (Persson & Erjefält 1982). Signs of CNS-stimulant behavioural effects such as restlessness, irritability and insomnia were not experienced by healthy volunteers given a wide range of i.v. and oral doses of enprofylline (IV). Tremor after theophylline is a frequently reported side effect (Rall 1980) and was also objectively verified in one study (Svedmyr & Svedmyr 1982). It has been suggested to be a sign of CNS-stimulation (VI and Rall 1980, Persson & Erjefält 1982).

Tremor of hands was assessed in the studies on asthma patients (V-VII). At largely equipotent bronchodilating doses of theophylline and enprofylline only theophylline produced significant tremor (VI). The dose levels chosen also gave minimum cardiovascular effects, which is of interest, since tremor recordings can be influenced by chro-

notropic effects (Thiringer & Svedmyr 1975). Furthermore, tremor of hands was not associated with still higher plasma enprofylline levels neither after oral (V) nor i.v. (VII) administration. It is suggested that the difference in tremorogenic action between enprofylline and theophylline may reflect their abilities to antagonize central nervous depressant actions of adenosine.

Other side effects. Anorexia, nausea and vomiting are associated with theophylline treatment and are roughly related to the plasma levels of the drug (Jenne et al. 1972, Jacobs et al. 1976). The emetic effect is probably exerted mainly by stimulation of the vomiting centre in the medulla oblongata (Borison & Wang 1953). On a molar basis enprofylline was slightly more prone to induce nausea in cats and dogs than theophylline (Persson & Erjefält 1982). In the studies on healthy subjects nausea was observed after about 25% of the drug administrations. In a total of 48 experiments on these drug-naive subjects nausea was accompanied by vomiting at two occasions (III-IV). Nausea seemed to be dependent of the plasma level of enprofylline. It occurred only after the higher doses given to the healthy subjects and was related to peak drug concentrations (IV). Nausea and vomiting also occurred in 3 patients with severe renal impairment and sustained high plasma concentrations of the drug (III). Out of 24 administrations in asthma patients only a few were associated with shortlasting nausea, among them one administration to the only asthma patient who was not on theophylline maintenance treatment (VI). Nausea may be a xanthine-induced effect pronounced preferentially in drug-naive subjects and may subside by continuous treatment (cf. Rall 1980). The present findings suggest that the nauseatic effect may be unrelated to adenosine antagonism (cf. Persson & Erjefält 1982).

Beside nausea, headache was the most common side effect in healthy volunteers, most frequently noted 3-5 h after the drug administrations, and not clearly related to the peak plasma concentrations. The headache was of the pulsating type making the vasodilator action of enprofylline a possible contributing factor. The limited number of subjects in the present study does not allow a quantitative conclusion about the incidence and severity of the side effects. Headache, nausea and flushing, that enprofylline has in common with theophylline, may be the dose-limiting adverse reactions, and are probably not related to adenosine antagonism. The adverse reactions of enprofylline were generally mild and short-lasting and seemed to be concentration dependent.

The adverse reactions in the studies on the healthy volunteers were therefore not considered a hindrance for further evaluation of enprofylline as a drug candidate and as a clinical pharmacological tool for elucidation of the physiological roles of adenosine.

GENERAL SUMMARY AND CONCLUSIONS

Enprofylline is a new xanthine derivative that in animal studies shows high oral absorption and is eliminated almost entirely by renal excretion of unchanged drug. Enprofylline is about 5 times more potent than theophylline as a bronchodilator and possesses antiallergic and anti-inflammatory activities in experimental animals. Enprofylline is a poor adenosine antagonist in the systems studied preclinically so far. This is in contrast to theophylline that is a potent adenosine antagonist.

In man enprofylline was well absorbed, negligibly metabolized and excreted unchanged in the urine with a high renal clearance. Volumes of distribution and protein bin-

ding were of the same order of magnitude as for theophylline. The pharmacokinetic profile in asthma patients was basicly not different from that in healthy subjects. Patients with renal failure showed an expected reduction of renal drug clearance and protein binding and also a reduction of non-renal clearance, which, however, may be age--related. The altered pharmacokinetics in uremia should require specific dose regimen adjustments in this patient cathegory.

Enprofylline produced marked and plasma concentration--dependent bronchodilation in asthma patients. The plasma concentration interval for bronchodilation, about 1-5 mg/L, suggests that the enprofylline/theophylline potency ratio of 5 / 1 is valid also in man. These results do not support an important role for adenosine antagonism in the bronchodilator effects of xanthines in man.

Enprofylline was well tolerated given orally and intravenously in bronchodilating doses. A minor side effect is occasional facial flushing. Headache, nausea and vomiting are probably the dose-limiting adverse effects, since they appear to be plasma concentration-dependent. The risk for a drop in blood pressure, occurring after rapid i.v. injections of theophylline, is likely to remain with enprofylline. These adverse effects are apparently not related to adenosine receptor antagonism.

Enprofylline differed from theophylline by lacking tremorogenic effects in man. This difference may be attributed to previously shown low ability of enprofylline and potent ability of theophylline to antagonize depressant actions of adenosine in the central nervous system.

ACKNOWLEDGEMENTS

The studies have been carried out at the departments of Clinical Pharmacology, University Hospitals of Lund and Göteborg, and at the department of Nephrology, University Hospital of Linköping.

I wish to express my sincere gratitude to:

My principal tutor, professor Karl-Erik Andersson, for his guidance, constructive criticism and friendly encouragement.

Dr Carl G A Persson for his enthusiastic supervision and support throughout the course of the investigations. It has been most instructive and stimulating to be his co-worker.

Dr Olof Borgå, for teaching me pharmacokinetics. His professionalism and common sense are most admirable.

Professor Nils Svedmyr, for generously providing me excellent working conditions. His hospitality is greatly appreciated.

Dr Rutger Larsson, who introduced me to the pharmacokinetics in renal insufficiency, for valuable advise and pleasant collaboration, which I hope will continue.

My other co-authors, Drs. Lars-Erik Edholm, Per-Olof Fagerström and Gunnar Kjellin, for expert advice and valuable discussions.

Dr Tore Mellstrand, for generously performing the theophylline analyses, and his wife Eva, for their hospitality during the studies in Göteborg.

Carl-Johan Lamm and Karin Larsén, for performing the statistical calculations.

Miss Lena Heintz and Mrs Christel Bodenäs for excellent performance of the enprofylline analyses.

Mrs Kerstin Tegnér and Miss Inger Svensson for expert management of the assays on protein binding.

Furthermore, I wish to express my sincere gratitude to:

Dr Nils Johannesson and Dr Jan Castenfors for valuable suggestions.

Ms Marie Carlsson, Helen Edbom, Birgitta Edlund, Anna-Britta Eriksson, Kerstin Lindmark, Gertrud Lundkvist, Lise-Lott Magnusson, Stina Ragnarsson, and Marianne Robertsson, for their skilful technical assistance and their concern for the subjects and patients.

Mrs Elin Owman, for the skilful final typing of this thesis.

Ms. Else-Britt Granbom, Ingegerd Källén, Inger Mårtensson, and Gunilla Sernelin, who so willingly, rapidly and excellently typed and retyped my manuscripts.

Elisabeth Lind and Stig Johnsson, for cover and illustrations.

Staff members of AB Draco, who in various forms gave help and advise during the studies.

Dr Bengt Bengtsson, for his support in the start of the clinical part of the enprofylline project, also being the first volunteer to take the drug. His friendship is greatly appreciated.

Last, but not least, my beloved wife, Marianne, who spared no trouble to help me perform these studies.

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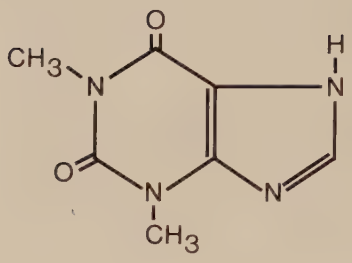
I

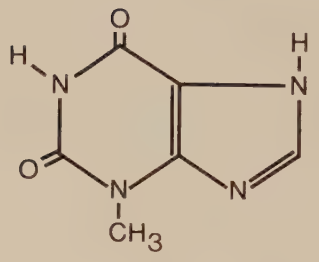
Pharmacokinetics of Enprofylline in Healthy Subjects after Intravenous Single Dose Administration

Olof Borgå, Karl-Erik Andersson, Lars-Erik Edholm, Per-Olof Fagerström, Erik Lunell and Carl G A Persson

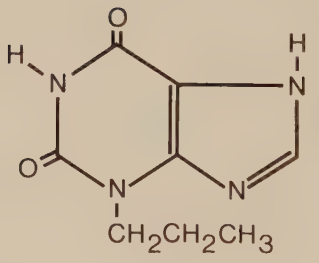
Abstract. The kinetics of enprofylline, a novel type of antiasthmatic xanthine derivative, have been studied. Eight healthy subjects recieved 3 different single doses of enprofylline, 0.5, 1.0 and 1.5 mg/kg, administered as an infusion over 10 minutes. Plasma and urine levels of unchanged enprofylline were followed up to 3-8 hours and 21-24 hours after dose, respectively, urine being collected in fractions. Volume of distribution terms V_{area} and V_{ss} were similar and averaged 0.57 and 0.51 liter/kg, respectively. Total clearance averaged 0.25 liter/hr/kg. Since the mean recovery of unchanged drug was 89 %, most of the total clearance was accounted for by renal clearance, which was 200-400 ml/min. The high renal clearance indicates that the major elimination route is by active tubular secretion. Plasma half-life varied between individuals from 1.2 to 1.9 hours. With the high performance liquid chromatography (HPLC) method used, urine levels could be followed for at least 8-9 half-lives. There were some indications of a slight dose dependency in CL and CL_{ren} that might be more pronounced at higher doses. However, at the investigated doses, the kinetics of enprofylline are best described by first-order kinetics. In contrast to theophylline, enprofylline was mainly eliminated by the renal route, which may be an advantage in its future clinical use as an antiasthmatic drug.



Theophylline



3-Methylxanthine



Enprofylline

Fig. 1. Structures of theophylline, 3-methylxanthine and enprofylline.

Enprofylline (pINN, Fig. 1) seems to be about 5 times more potent than theophylline (Fig. 1) as a bronchodilator in both animals[15,18] and asthma patients[8]. Despite the chemical similarities between the two compounds, enprofylline exhibits a profile of action that is different from that of theophylline. For example, in a series of animal studies, enprofylline has been shown to lack the diuretic[14,15] and CNS-stimulant behavioural effects of theophylline[13,14,16]. This may well be explained by the finding that enprofylline has no or negligible ability to antagonise adenosine in different tissues where theophylline behaves as a potent adenosine receptor antagonist[11,16]. At high plasma levels, theophylline can induce life-threatening seizures[2,13]. Too high or too low plasma levels of theophylline may be caused by its wide and unpredictable variability in rate of metabolism[4,7,10,19]. The structural resemblance of enprofylline with 3-methylxanthine (3-MX, Fig. 1), an active metabolite of theophylline[1,5,12,17,22], suggests that enprofylline like 3-MX[6] would be mainly excreted unchanged in the urine[14,18]. This possibility is validated by the present study which is the first to evaluate the pharmacokinetics of enprofylline in humans. The investigated doses, 0.5, 1.0 and 1.5 mg/kg were in the anticipated range for the clinical utilization of the compound as an antiasthmatic drug. The main aims of the pharmacokinetic study were to calculate basic kinetic parameters of the drug and their interindividual variability, and to estimate relative contributions of renal and nonrenal elimination routes.

Methods

Subjects. Eight male subjects aged between 24 and 34 years took part in the study after informed consent. (For anthropometic data, see Table I). They were healthy according to physical examination and standard blood and urine laboratory tests. The study protocol was approved by the Ethical Comittee of the University of Lund.

Table I. Subjects and doses

Subject No.	Age	Height (cm)	Weight (kg)	Nominal dose ($mg \cdot kg^{-1}$)	Actual dose ($mg \cdot kg^{-1}$)
1	23	176	64	0.5	0.503
				1.0	1.126
				1.5	1.489
2	24	186	82	0.5	0.516
				1.0	1.037
				1.5	1.555
3	23	190	78	0.5	0.503
				1.0	1.017
				1.5	1.518
4	24	189	76	0.5	0.501
				1.0	0.997
				1.5	1.521
5	28	186	72	0.5	0.485
				1.0	0.976
				1.5	1.526
6	30	183	68	0.5	0.514
				1.0	0.969
				1.5	1.499
7	34	194	94	0.5	0.552
				1.0	1.076
				1.5	1.575
8	28	185	71	0.5	0.510
				1.0	1.034
				1.5	1.484

Drug administration. Each subject received three doses of enprofylline given at three different occasions 2 weeks apart (occasionally one week). The drug was administered by i.v. infusion of a water solution of enprofylline (pH 9.5) into an antecubital vein during 10 minutes using a Tekmar 922 infusion pump (Tekmar Medical Ltd, England).

Sterile solutions of 10 mg/ml of enprofylline in ampoules were diluted with saline just prior to injection to produce dose volumes large enough to be precisely delivered by the infusion pump. Since this dilution was performed using ordinary disposable syringes of relatively low accuracy, the obtained concentrations of enprofylline were measured in each solution, and the actual doses calculated (see Table I).

The subjects had their regular breakfast at least one hour before the dose and had lunch 3 hours after the dose. No coffee, tea, cola drinks or alcohol was permitted during the investigation days. Further details on investigational procedures are presented elsewhere[9]. Blood samples (5 ml) were drawn from an antecubital vein of the opposite arm via an indwelling catheter (Venflon(R)) at the following time-points: 5, 10, 15, 20, and 30 minutes, and 1, 2, 3, 5, and 7 hours after the start of the infusion. Heparinized tubes Venoject(R) were used, and plasma prepared by immediate centrifugation (within 2 minutes) of the samples. Urine was collected quantitatively in fractions (n = 14-17) up to 21-24 hours after dose. In general the over-night fraction was 9 hours. Urine volumes were determined by weighing, the weights being translated to volumes assuming a specific gravity of 1.02 g/ml. Plasma and urine samples were kept at -20°C until analysis. Storage at this temperature for several months and repeated freezing and thawing of the samples has no effect on the analytical results*.

Analysis of enprofylline in human plasma and urine. A detailed description of the analytical procedures used for determination of unchanged enprofylline in plasma and urine will be presented elsewhere*.

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*Edholm and Heintz, unpublished data.

Enprofylline was analyzed by high performance liquid chromatography (HPLC), employing columns packed with a chemically bonded reversed-phase material of the C_{18}-type.

A mobile phase consisting of methanol/water 30/70 (v/v) containing one gram of phosphoric acid per litre was used for plasma analysis. For urine the mobile phase consisted of acetonitrile, tetrahydrofurane and 0.005 mol l^{-1} phosphoric acid buffer pH 7.5 (6/2.5/94, v/v/v).

Plasma analysis. To 100 µl plasma was added an internal standard (3,7-dihydro-1-ethyl-3-(2-hydroxypropyl)-1H-purine--2,6-dione). The sample was made alkaline and then extracted for five minutes with a mixture of chloroform/isopropanol. After centrifugation the organic phase was discarded. Perchloric acid was added to the aqueous phase to precipitate residual proteins. After centrifugation the sample was injected onto the chromatographic column.

Urine analysis. After the addition of internal standard (same as with plasma) to 100 µl urine, the sample was diluted ten times with either water or mobile phase before injection onto the column.

Recovery and precision. The absolute recoveries of enprofylline and its internal standard were complete (100%) in urine and only slightly lower in plasma (94 and 96%, respectively). The data on inter assay precision varied at different concentrations and between plasma and urine but were in no instance worse than 3.5% expressed as coefficient of variation.

Infusion solution analysis. For analysis of the diluted infusion solution a mobile phase of acetonitrile, tetrahydrofurane and phosphate buffer, pH 6.0 (15/0.5/85, v/v/v) was used to decrease retention times. Since the above internal standard was incompletely separated from enprofylline with this mobile phase, we used as internal standard 3,7-dihydro--3-(2-methylpropyl)-1H-purine-2,6-dione. Quantitation was performed using calibration samples of the same principal composition as the infusion solutions.

Pharmacokinetic calculations. Area under plasma concentration-time curves (AUC) were determined by the trapezoidal rule. AUC from the last plasma concentration, C_n, to infinity

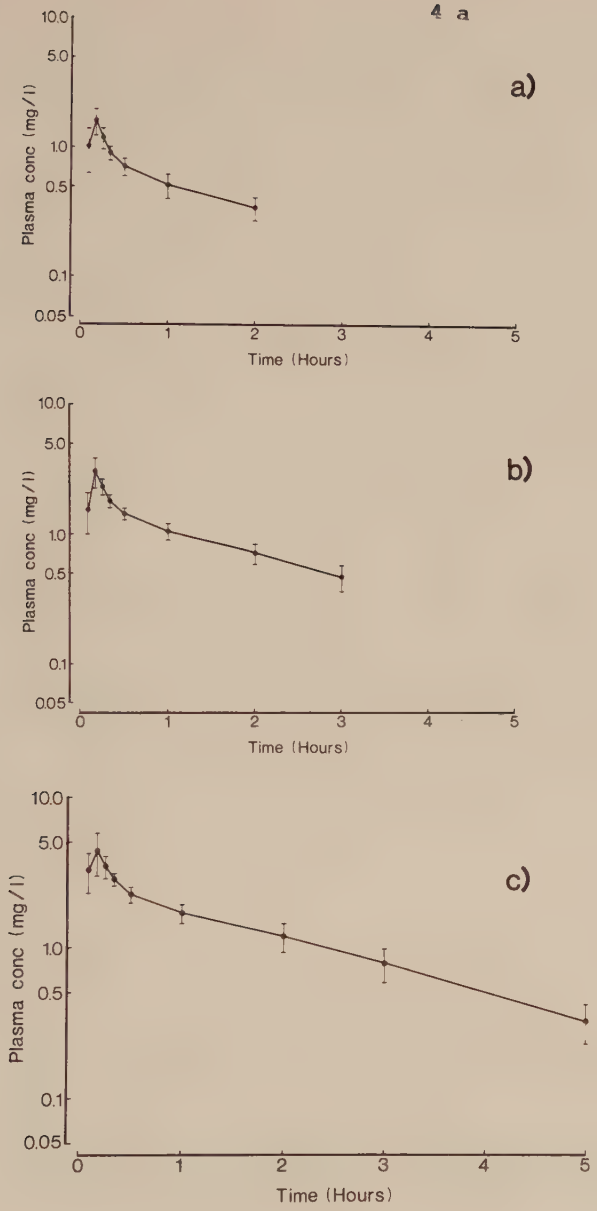

Fig. 2. Mean plasma concentrations of enprofylline in 8 healthy subjects after 10 min i.v. infusion. Bars indicate ±SD. a) Dose 0.5 mg/kg. b) Dose 1.0 mg/kg. c) Dose 1.5 mg/kg.

was estimated as C_n/β. Beta was estimated as the slope of the apparently linear last portion of the ln c_p *vs* time curves, $t_{½}$ as ln 2/β. Beta and $t_{½}$ were also estimated from the excretion rate *vs* time curves excluding the last (over-night) sample, owing to the difficulty to assign an accurate sampling midpoint to this large fraction. Total plasma clearance, CL was estimated as Dose/AUC, and volume of distribution V_{area} or V_β during the beta elimination phase as CL/β. Volume of distribution steady-state V_{ss}, was calculated as described by Benet and Galeazzi[3] and Smith et al.[20]:

$$V_{ss} = \frac{\text{Dose} \cdot \text{AUMC}_\infty}{[\text{AUC}_\infty]^2} - \frac{\text{Dose} \cdot \text{Infusion time}}{2 \cdot \text{AUC}_\infty}$$

Here $AUMC_\infty$ is the area under the first moment of the plasma curve, i.e., $\int_0^\infty t \cdot c_p \cdot dt$.

Average renal clearance during the complete experiment was estimated as total recovery of unchanged enprofylline in urine divided by AUC_∞, while renal clearance during each interval was calculated as the urinary recovery during that particular period divided by AUC during the same period.

Statistics. Paired dependent Student t-test was used to test differences in parameters obtained at the three doses.

Results

In general the plasma fall-off curves were log-linear from one hour on, but in many cases also the 30 minutes point fell on the same line. Mean data of all subjects are presented in Fig. 2. The side-effects observed are discussed in detail elsewhere[9].

Recoveries of unchanged enprofylline in urine were generally high, and varied between individuals from 83.8 to 93.9% (Table II). The average recoveries were 89.9 (n = 7), 86.5 (n = 6) and 89.8 (n = 7)% at the doses 0.5, 1.0, and 1.5 mg/kg, respectively. Large variations in recovery between the doses were found only in one subject (No. 4). A closer inspection of values of urine flow rates, excretion rates, and renal clearance in the various sampling intervals revealed values

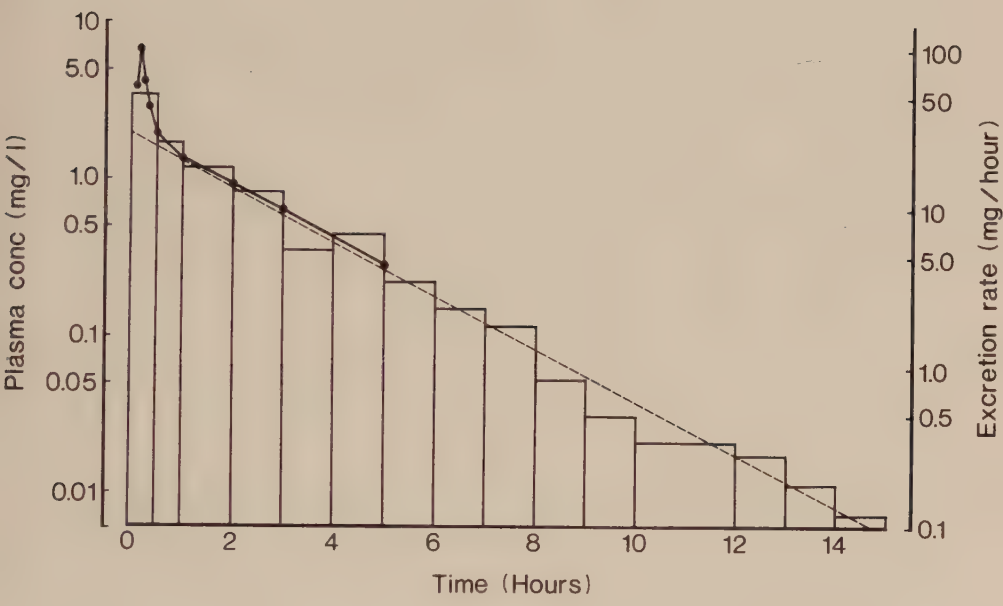

Fig. 3. Plasma concentrations (filled circles) and excretion rates in urine (bars) of enprofylline in one subject (No. 6) after 10 min i.v. infusion of 1.5 mg/kg. Note the two different scales on the ordinate axis. A straight line was fitted to the mid timepoints of the excretion rate bars by linear regression analysis, starting with the second fraction (30-60 min).

outside what was considered normal variation, indicating poor compliance with sampling instructions and/or erroneous volumetric measurements in this subject. Excretion data from subject No. 4 were therefore considered less reliable and were not used in further calculations.

Renal clearance values were high, 200-400 ml/min (Table II). Variations between different doses within each individual were generally moderate. The mean renal clearance at 1.5 mg/kg, 262 ml/min, was significantly ($p < 0.05$) lower than that at 0.5 mg/kg, 300 ml/min.

Biological half-lives calculated from the slope of excretion rate vs time curves were essentially the same at the three doses, the average values being 1.71 (0.5 mg/kg), 1.66 (1.0 mg/kg), and 1.70 (1.5 mg/kg) hours (Table III).

Average half-lives from plasma data were only slightly shorter; 1.59, 1.63 and 1.64 hours, respectively. Half-lives obtained from plasma and urine data for the two higher doses correlated well (Figs. 3,4). When data from the lowest dose were included, the correlation coefficient deteriorated (Fig. 4). This was probably due to the fact that plasma levels after this dose could be followed for a shorter time period; in three subjects only for two hours, in another three for three hours, and in the remaining two subjects for five hours. In spite of the slight inaccuracies in determining plasma half-life of the lowest dose being indicated by the above results, we did not find it justified to use the values obtained from urine data in our further calculations on the plasma data. Thus total clearance (CL), V_{area} and V_{ss} in Table III are based on plasma data only.

AUC values up to 2 hours, normalized for differences in dose (Table IV), were slightly elevated (about 8%) at the highest dose level (1.5 mg/kg) compared to the middle dose level (1.0 mg/kg, $p < 0.01$) but not compared to the lowest dose level. Also total clearance values (Table III) were slightly (about 9%) but significantly decreased for the highest dose compared to the middle dose ($p < 0.05$). Since this indicated non-linear kinetics, it might not be absolutely correct to include data for the highest dose when calculating mean data in each individual and in the whole group of the kinetic

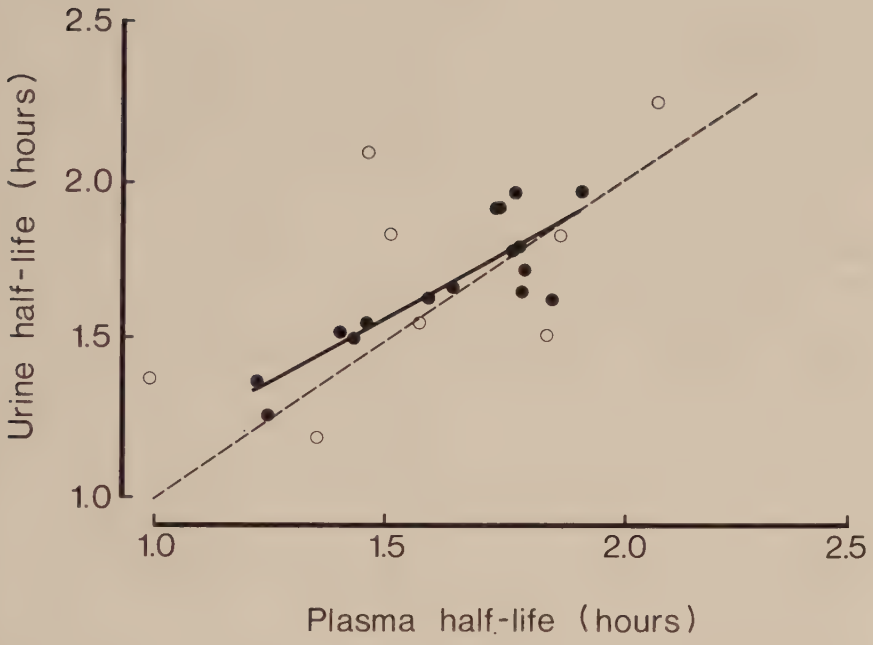

Fig. 4. Relationship between half-lives calculated from plasma concentrations and urinary excretion rates.
o - o 0.5 mg/kg, ● - ● 1.0 and 1.5 mg/kg.
Regression line (not shown) for all points:
$y = 0.55 + 0.70x$ ($r = 0.70$, $t = 4.62$).
Regression line for the two higher doses (solid line):
$y = 0.35 + 0.81x$ ($r = 0.85$, $p = 0.000025$).
Broken line: $y = x$.

parameters CL, V_{area} and V_{ss} as done in Table III. However, this was done to provide figures of these parameters useful in clinical applications, since plasma levels after therapeutic doses will probably be in the order of 1-5 µg/ml[8].

Discussion

The distribution phase of enprofylline was rather short and appeared from plasma data to be complete within one hour. Urine levels could be followed much longer than plasma levels by the employed analytical method. Nevertheless, half-lives from urine data correlated well with plasma half-lives and were only slightly longer. In the almost perfectly linear log excretion *vs* time plots there was no indication of more than one elimination phase. This would rule out the possible existence of "deep compartments" of any significant size. Enprofylline, in similarity to theophylline, does not appear to be a typical two- or multicompartment drug. In line with this notion is the fact that values of V_{area} and V_{ss} are similar (Table III).

The main portion of the dose was excreted *via* the kidney. No metabolites of enprofylline have so far been identified but the average 89.1% recovery of the dose in a 24-hours urine leaves some 10% to be eliminated by non-renal routes. Biliary excretion and subsequent fecal elimination should be considered as one possible elimination route, at least theoretically. However, since enprofylline is rapidly absorbed from the gut, biliary excretion would inevitably lead to enterohepatic recycling of the drug. This is contradicted by the smooth plasma curves and the short half-life. Thus biotransformation would be the most likely alternative to account for the missing 10%.

The renal clearance of enprofylline was high, 200-400 ml/min, which is similar to the 170-233 ml/min reported for 3-methylxanthine (3-MX)[21], the active metabolite of theophylline[17]. No metabolism of 3-MX has been observed[6], which further underlines the similarity between the two compounds.

Table II. Renal clearance[1] and fraction of dose excreted unchanged in urine (recovery)

Subject No.	Nominal dose ($mg \cdot kg^{-1}$)	Recovery (%)	Mean	CL_{renal} ($ml \cdot min^{-1}$)	Mean
	0.5	86.8		331	
1	1.0	78.9	84.6	275	303
	1.5	88.0		303	
	0.5	97.4		262	
2	1.0	(78.7)[2]	93.9[3]	- [4]	265[3]
	1.5	90.4		268	
	0.5	82.1		229	
3	1.0	83.0	84.8	228	229
	1.5	89.4		231	
	0.5	(64.9)			
4	1.0	(99.6)	-[4]	- [4]	- [4]
	1.5	(71.7)			
	0.5	90.1		292	
5	1.0	92.4	91.6	322	296
	1.5	92.3		273	
	0.5	93.0		324	
6	1.0	93.6	92.4	307	296
	1.5	90.9		256	
	0.5	94.9		407	
7	1.0	88.5	92.3	322	344
	1.5	93.5		303	
	0.5	84.8		258	
8	1.0	82.8	83.8	206	221
	1.5	83.9		200	
	0.5	89.9(n=7)	89.1	300(n=7)	279
Mean	1.0	86.5(n=6)	SD 4.2	277(n=6)	SD 44
	1.5	89.8(n=7)		262(n=7)[5]	

1) Calculated as total recovery in urine divided by AUC_{∞}
2) One sample was lost but was estimated from actual plasma concentration and renal clearance to account for another 10.7% of the dose. Thus total recovery was about 89.4%.
3) Based on 2 values.
4) Not calculated because of unreliable sampling.
5) Significantly lower than at 0.5 mg/kg ($p<0.05$).

The present study was not designed to evaluate the mechanisms involved in the renal elimination of enprofylline. However, since its average binding in human plasma is about 47%*, only about 50-80 ml/min of the renal clearance may be accounted for by glomerular filtration. Therefore, active tubular secretion must account for at least 120-320 ml/min. Whether tubular reabsorption occurs in addition to the two other processes cannot be evaluated from the present data. It has to be studied under conditions where urine flow and pH are better controlled.

Active tubular transport is in principle capacity limited. Therefore a dose dependent decrease in renal elimination has to be anticipated at some stage of dose level. The data of the present study actually indicate a decrease in total and renal clearance values at the highest dose, but the effects were small. Probably the doses used were not high enough to demonstrate unequivocally any dose dependency. However, data obtained after higher oral doses show a disproportionate increase in AUC with increasing doses**. Nonlinear or dose dependent elimination has been demonstrated with therapeutic doses of theophylline, but in this case it is caused by capacity limited metabolism[21].

The apparent volume of distribution, V_{area}, was similar to that of theophylline. Since figures of protein binding in plasma were almost identical*, this indicates a similar degree of over-all tissue distribution of the two drugs. As mentioned above the two drugs differ considerably in other respects. Thus theophylline is eliminated mainly (about 90%) by metabolism. In terms of interindividual variability in drug response, this may be a disadvantage, since rate of metabolism may vary considerably between subjects, and there is no universal method to assess an individual's drug metabolizing capacity. Such tests are readily available for drugs that are eliminated renally, creatinine clearance being the most widely accepted. The present study supports the notion that it could be of value to produce a xanthine drug that is little

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* Tegnér, Borgå and Svensson, unpublished data.

**Lunell et al., unpublished data.

Table III. Kinetic parameters derived from plasma concentrations or urinary excretion rates

Subj. No.	Nominal dose ($mg \cdot kg^{-1}$)	$t_{½}$ (hr)	Mean	V_{area} ($liter \cdot kg^{-1}$)	Mean	Plasma data: V_{ss} ($liter \cdot kg^{-1}$)	Mean	CL ($liter \cdot hr^{-1} \cdot kg^{-1}$)	Mean	Urine excretion data: $t_{½}$ (hr)	Mean
1	0.5	0.99		0.51		0.41		0.358		1.38	
	1.0	1.46	1.23	0.69	0.59	0.62	0.51	0.327	0.336	1.55	1.40
	1.5	1.24		0.58		0.49		0.322		1.26	
2	0.5	1.85		0.53		0.46		0.197		1.52	
	1.0	1.77	1.80	0.58	0.56	0.50	0.50	0.227	0.214	1.78	1.65
	1.5	1.79		0.56		0.53		0.217		1.66	
3	0.5	1.57		0.49		0.43		0.214		1.56	
	1.0	1.59	1.52	0.48	0.46	0.43	0.41	0.211	0.208	1.64	1.58
	1.5	1.40		0.40		0.38		0.199		1.53	
4	0.5	2.09		0.65		0.59		0.217		2.26	
	1.0	1.86	1.91	0.63	0.60	0.58	0.55	0.236	0.219	1.63	1.95
	1.5	1.78		0.53		0.48		0.205		1.97	
5	0.5	1.35		0.52		0.46		0.270		1.19	
	1.0	1.22	1.33	0.51	0.51	0.46	0.46	0.290	0.269	1.37	1.36
	1.5	1.43		0.51		0.45		0.246		1.51	
6	0.5	1.88		0.83		0.71		0.307		1.84	
	1.0	1.64	1.77	0.69	0.72	0.57	0.60	0.290	0.282	1.67	1.77
	1.5	1.79		0.64		0.52		0.248		1.80	
7	0.5	1.51		0.60		0.57		0.274		1.84	
	1.0	1.73	1.72	0.58	0.58	0.54	0.55	0.232	0.238	1.92	1.91
	1.5	1.92		0.57		0.54		0.207		1.98	
8	0.5	1.46		0.54		0.53		0.257		2.10	
	1.0	1.8	1.67	0.55	0.53	0.52	0.51	0.211	0.223	1.73	1.92
	1.5	1.75		0.50		0.49		0.200		1.92	
Mean	0.5	1.59		0.58		0.52		0.262		1.71	
	1.0	1.63	1.62	0.59	0.57	0.53	0.51	0.253	0.249	1.66	1.69
	1.5	1.64	SD	0.54	SD	0.49	SD	0.231	SD	1.70	SD
			0.24		0.08		0.06		0.044		0.23

metabolised[18]. The renal elimination of enprofylline may therefore be a great advantage in the clinical situation when it comes to deciding the optimal individual dosage.

Acknowledgment. We would like to thank Miss Lena Heintz and Mrs Christel Bodenäs for skilful technical assistance.

Table IV. Comparisons of areas[1] under the plasma curve (AUC) of various doses

Dose ($mg \cdot kg^{-1}$) / Subj No.	AUC[1] ($mg \cdot liter^{-1} \cdot hr$)			Normalized[2] AUC ($mg \cdot liter^{-1} \cdot hr$)	
	0.5	1.0	1.5	0.5	1.0
1	1.11	2.14	3.22	3.28	2.83
2	1.47	2.65	3.78	4.44	3.85
3	1.43	2.83	4.59	4.31	4.23
4	1.18	2.26	4.05	3.59	3.44
5	1.20	2.34	3.94	3.78	3.66
6	0.97	2.10	3.63	2.84	3.25
7	1.22	2.58	3.84	3.46	3.77
8	1.18	2.58	3.91	3.43	3.70
Mean			3.87	3.64	3.59
SD			0.39	0.53	0.42

p=0.18

p=0.0070

1) Area under curve from 0 to 2 hrs.

2) AUC multiplied by actual dose factor for that experiment, e.g. (dose in 1.5 $mg \cdot kg^{-1}$ experiment): (dose in 0.5 $mg \cdot kg^{-1}$ experiment)

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II

Pharmacokinetics of Enprofylline in Healthy Subjects After Oral Single Dose Administration

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P.-O. Fagerström , G. Kjellin and C.G.A. Persson

Abstract: Enprofylline, a new potent bronchodilator xanthine with a seemingly beneficial pharmacodynamic profile, was given orally as a water solution to six healthy subjects in single doses of 2, 4 and 6 mg/kg. The two lower doses produced plasma concentrations in the range 1-4 mg/L, i.e. in the assumed "therapeutic interval" according to previous animal studies. Recovery of unchanged drug averaged about 90% in urine collected for 24 h, indicating high oral absorption and negligible metabolism. A prolongation of the mean absorption time was recorded at the two higher dose levels, up to 1.15 ± 0.10 and 1.60 ± 0.22 hours after 4 and 6 mg/kg enprofylline, respectively, compared to 0.58 ± 0.10 hours after 2 mg/kg. Multiple peaks in the plasma concentration-time curves at these dose levels indicate intermittent and delayed gastric emptying. The areas under the plasma concentration-time curves (AUC) increased more than proportional to dose, and bioavailability calculated as AUC_{oral}/ /$AUC_{i.v.}$ attained values above 100% (average 114%) at the 6 mg/kg dose. The data indicate capacity limited elimination.

Key words: Enprofylline - absorption - bioavailability - healthy subjects - oral administration - pharmacokinetics - single-dose.

Table 1

Data on the healthy volunteers

Subject No	Age (years)	Weight (kg)	Height (cm)
1	23	64	176
2	24	82	186
3	23	78	190
5	28	72	186
6	30	68	183
7	34	94	194

Theophylline is a drug of considerable importance in the treatment of asthma, when due consideration has been taken to its variable rate of metabolism [5,7], narrow therapeutic index [2] and potentially serious side effects [3]. Some of the latter are possibly related to adenosine receptor antagonism [11]. The main active metabolite of theophylline, 3-methylxanthine, is not further metabolized and is rapidly eliminated by urinary excretion [4]. 3-Methylxanthine possesses a bronchodilating potency that is 1/5 to 1/3 of that of theophylline [9]. In the search for new antiasthmatic xanthine derivatives, 3-propylxanthine, enprofylline, INN, in preclinical studies was shown to lack adenosine antagonistic ability [11], and to be 5 times more potent as a bronchodilator than theophylline [12]. Furthermore, enprofylline given orally to rats was completely absorbed and eliminated almost solely by renal excretion [10].

The present study describes the first oral administration of enprofylline to man. The aim of the study was to obtain human pharmacokinetic data on enprofylline when given orally as a water solution in single doses at three different dose-levels. Tolerability data obtained in the study will be reported elsewhere (Lunell et al. unpublished).

Material and Methods

Study design. The experiments were performed as an open study of increasing doses of enprofylline with a wash-out period of 2 weeks between the trials. The choice of doses was based on the potency ratio for enprofylline/theophylline obtained preclinically. Data from parallel studies of i.v. administration (0.5, 1.0 and 1.5 mg/kg) in the same subjects (Borgå et al. unpublished) allow conclusions to be drawn regarding rate and completeness of absorption.

Subjects. Of the 8 healthy male volunteers who took part in the i.v. study, five completed the present study. Subject Nos. 4 and 8 in the i.v. study (numbers are according to the i.v. study) refrained from participating in the present study, and subject No. 1 declined to take the highest oral dose. In all cases the decisions were due to personal reasons and not caused by side-effects in the previous experiments. The mean age of the subjects was 27 years (range 23-34 years) and the mean weight was 76 kg (range 64-94 kg). They were healthy according to medical history, a physical examination and routine laboratory tests. Xanthine-containing food and beverages were not permitted during 12 hours before and 24 hours after ingestion of the drug. The subjects were allowed a light breakfast not later than 1 hour before the start of the trial.

Procedure. A water solution (450 mL) containing only enprofylline was given orally in doses of 2 mg/kg (10.3 umol/ /kg, 4 mg/kg (20.6 µmol/kg) and 6 mg/kg (30.9 µmol/kg). Blood samples (5 mL) were collected from an antecubital vein at 10, 20, 30, 40, 50, 60, and 90 min and 2, 3, 4, 5, 6, 8, and 10 hours after drug administration for the measurement of plasma levels of enprofylline. Blood samples were drawn via an indwelling catheter (Venflon^{R}). Heparinized tubes (Venoject^{R}) were used, and plasma was prepared by centrifugation of the samples. Urine was collected quantitatively in fractions (n = 8) up to 24 hours after dose. The over--night fraction was 12 hours. After sampling, the urine fractions were pooled and samples were collected also from the urine pools. The samples of plasma and urine were frozen within 1 hour and stored at -20°C until analysed. Urine fractions were weighed, and corresponding volumes calculated assuming a specific gravity of 1.02 g/mL.

Blood pressure, heart rate and ECG were monitored, and orthostatic tests were performed. The subjects were regularly questioned about symptoms that could be attributable to the drug. They remained supine for the first 4 hours,

except for the orthostatic tests, and were then in normal daily activity in the laboratory.

Analysis of enprofylline in plasma and urine. For determination of unchanged enprofylline in plasma and urine liquid column chromatography was used (Edholm & Heintz, unpublished), employing columns packed with a chemically bonded reversed-phase material of C_{18}-type. A mobile phase consisting of methanol/water 30/70 (v/v) containing 1 gram of phosphoric acid per litre was used for plasma analysis. For urine the mobile phase consisted of acetonitrile, tetrahydrofurane and 0.005 M phosphoric acid buffer, pH 7.5 (6/2.5/94, v/v/v).

Plasma analysis. To 100 µl plasma was added an internal standard (3,7-dihydro-1-ethyl-3-(2-hydroxypropyl)-1H-purine--2,6-dione). The sample was made alkaline and then extracted with a mixture of chloroform/isopropanol. After centrifugation the organic phase was discarded. Perchloric acid was added to the aqueous phase to precipitate residual proteins. After centrifugation, the sample was injected onto the chromatographic column.

Urine analysis. After the addition of internal standard (the same as with plasma) to 100 µl urine, the sample was diluted ten times with either water or mobile phase before injection onto the column.

Sensitivity and precision. The sensitivity of the method permitted determination down to 0.1 mg/L. The inter assay precision varied at different concentrations and between plasma and urine. It was always less than 3.5% at 0.4 and 3.0 mg/L, expressed as coefficient of variation.

Statistical methods. Mean values are given together with standard error of the mean (SEM) in the text.

Pharmacokinetic calculations

Rate of absorption. The rate of absorption, expressed as the mean absorption time, MAT [1], was calculated as

$$MAT = MRT_{oral} - MRT_{i.v.} \qquad (1)$$

The mean residence time, MRT, or the mean transit time, obtained in the present study and in the parallel i.v. study in the same subjects (Borgå et al. unpublished) were used for the calculations of MAT. MRT is defined [8] as

$$MRT = \frac{\int_0^\infty t \cdot Cdt}{\int_0^\infty Cdt} \qquad (2)$$

Rate of absorption was also calculated according to Wagner & Nelson [13]. This method assumed that a one-compartment model can be applied. In the case of enprofylline, this is only true after completed distribution phase, i.e. from 0.5 h after i.v. administration (Borgå et al. unpublished). An expression relating the cumulative amount of drug absorbed $(X_A)_T$ from time zero to time T can then be generated, based upon the plasma concentration of the drug (C_T) and the elimination rate constant K_E [13]:

$$\frac{(X_A)_T}{V} = C_T + K_E \cdot \int_0^T Cdt \qquad (3)$$

where V is the apparent volume of distribution.

To calculate the right-hand side of the equation, we used for K_E the mean of each individual's elimination rate constant (β) obtained in 3 experiments at different dose-levels in the i.v. study (Borgå et al. unpublished). Since V is unknown, the wished amount absorbed vs time curve is actually obtained in concentration units. It nevertheless gives an informative graphic presentation of the absorption kinetics. If, e.g., absorption is fast enough, the 2-compartment

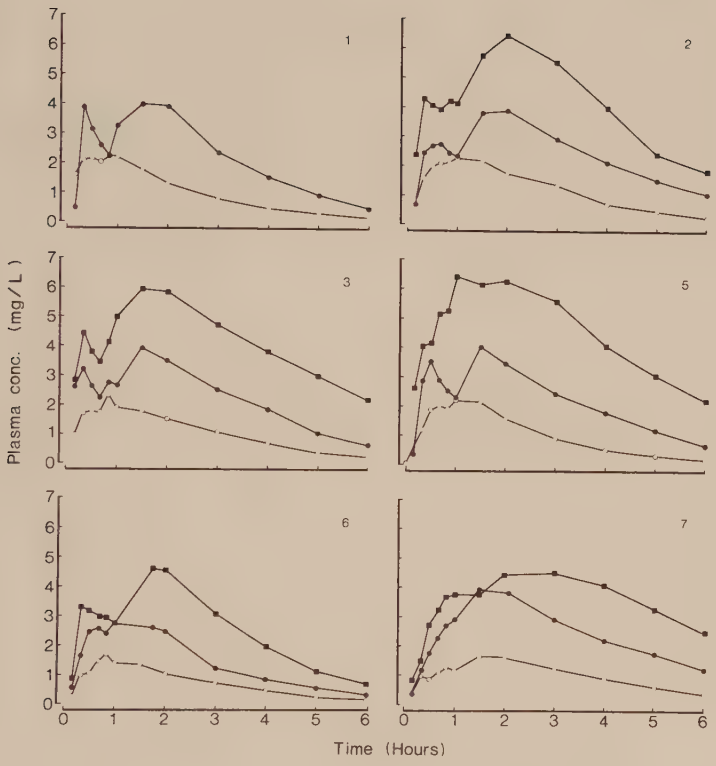

Fig. 1. Plasma concentration curves in six subjects after oral administration of enprofylline.
o—o 2 mg/kg; ●—● 4 mg/kg; ■—■ 6 mg/kg

characteristics of the drug will become apparent. Subsequent distribution from the central to the peripheral compartment will then show up as a notch in the curve.

Extent of absorption. The extent of absorption was determined by comparing the areas under the plasma concentration-time curves (AUC^{∞}) with those obtained after the 1 mg/kg intravenous dose in the i.v. study. The area up to the last concentration measured (AUC_t) was determined by the trapezoidal method and for the estimation of the area from the last data point (C_n), the elimination rate constant (β) of the 1 mg/kg i.v. dose was used

$$AUC^{\infty} = AUC_t + C_n/\beta \qquad (4)$$

Oral bioavailability (F) in per cent was calculated as

$$F = \frac{AUC^{\infty}_{oral} \cdot D_{i.v.} \cdot 100}{AUC^{\infty}_{i.v.} \cdot D_{oral}} \qquad (5)$$

where D is the dose.

Oral bioavailability (%) was also calculated as the ratio oral/i.v. urine recovery x 100. The mean of the recovery calculated from the cumulative urine fractions and from the urine pool was used. The mean of the urine recovery after the i.v. doses of 0.5, 1, and 1.5 mg/kg was used for comparison.

The pharmacokinetic parameters in the intravenous and the present study were calculated with the aid of a program run on an HP-9835 desk computer.

Results

Oral doses of enprofylline 2, 4 and 6 mg/kg produced peak plasma levels within 3 hours. The mean peak levels increased with increasing dose from 1.92 ± 0.16 mg/L following the 2 mg/kg dose to 3.69 ± 0.22 mg/L and 5.48 ± 0.42 mg/L

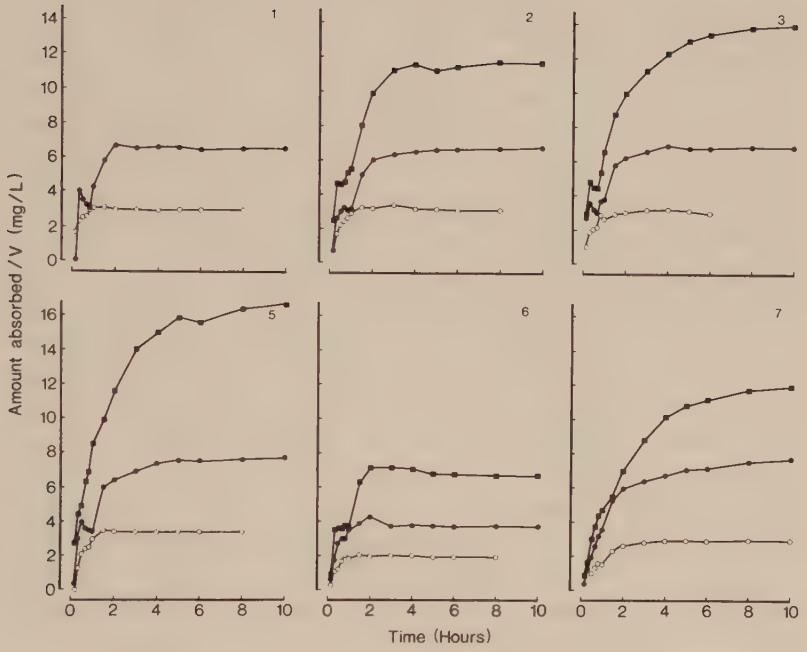

Fig. 2. Rate of drug absorption illustrated by Wagner-Nelson plots of plasma concentration data obtained in six subjects after oral administration of enprofylline. The elimination rate constants were mean values from experiments with three i.v. doses (0.5, 1.0 and 1.5 mg/kg) (Borgå *et al.* unpublished).
o—o 2 mg/kg; ●—● 4 mg/kg; ■—■ 6 mg/kg

following the 4 and 6 mg/kg doses, respectively. The individual enprofylline peak levels showed some variation and were for the 2 mg/kg dose as low as 1.66 mg/L and for the 6 mg/kg dose as high as 6.42 mg/L (Fig. 1). The mean absorption time (MAT) increased with increasing dose from 0.58 ± 0.10 hours after 2 mg/kg to 1.15 ± 0.17 hours (Table 2) and 1.60 ± 0.22 hours after 4 and 6 mg/kg enprofylline, respectively. An initial rapid increase in plasma drug concentration was followed by a double peak in subjects 1, 2, 3, 5, and 6 at the higher dose levels (Fig. 1).

From the Wagner-Nelson curves (Fig. 2) can be seen that the absorption is terminated 1-2 hours after the 2 mg/kg dose but still continuing 6 hours after the higher doses in subjects 3, 5 and 7. Notches in the curves (e.g. the 4 mg/kg dose in subject 1) indicate instances where a rapid absorption process has been suddenly interrupted.

The areas under the plasma concentration-time curves (AUC^{∞}_{oral}) showed a non-linear increase with increasing dose (Fig. 3). Bioavailability calculated as $AUC^{\infty}_{oral}/AUC^{\infty}_{i.v.}$ reached values > 100% at the 6 mg/kg dose level. In contrast, urinary recovery of unchanged enprofylline fell from 102.8 to 93.6% of that of the i.v. dose when the oral dose increased from 2 to 6 mg/kg (Table 3).

Discussion

The present study describes the first oral administrations of enprofylline to man. The two lower dose levels, 2 and 4 mg/kg, gave plasma levels in the range 1-4 mg/L. This is the assumed "therapeutic interval" as judged from preclinical data where a potency ratio of 5/1 was obtained for enprofylline relative to theophylline [12]. Subsequently it was shown in asthmatic patients that levels above 1 mg/L were associated with a significantly increased forced expiratory volume [6].

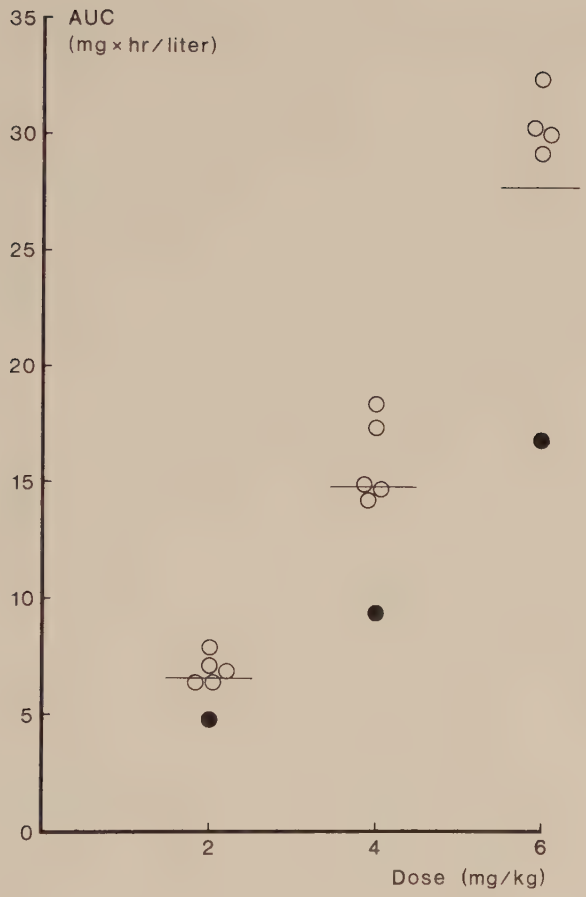

Fig. 3. Non-linear increase of the area under the plasma concentration-time curve (AUC^{∞}) with increasing dose. Filled circles denote subject No 7. Horizontal lines indicate arithmetic mean values of all subjects including No 7 (n = 6 exept for the highest dose where n = 5).

The relative rate of absorption of different oral doses can be estimated by comparing their peak concentrations and the times required to reach the peak. In the present study this method was difficult to apply because of multiple peaks. These were probably caused by variations in gastric emptying (Fig. 1). Mean absorption time (MAT) appears to be the ideal parameter to describe the absorption in a situation like this, since it is independent of the shape of the plasma concentration curve or the occurrence of double or multiple peaks. MAT is the mean time which a drug molecule spends at the input side before being absorbed [1]. MAT is the difference between the oral and the i.v. mean residence time (MRT). MRT is a composite of all kinetic processes including absorption and elimination.

In a cross-over study, in which elimination rate is assumed to be constant, differences in the MRT between the i.v. reference dose and the oral administrations reflect the absorption rates. This assumption is probably valid for enprofylline, since previous data indicate relatively small day-to-day changes in enprofylline clearance within the same individual (Borgå et al. unpublished). The calculation of MAT assumes linear kinetics. This prerequisite may not be absolutely fulfilled as discussed below, but the implied inaccuracy is probably small in comparison with the differences in MAT that were observed between the three oral doses.

The Wagner-Nelson method of calculating the amount absorbed [13] offers a potential advantage to other methods of evaluating absorption processes since it allows the simultaneous comparison of both rate and extent of absorption from the graph (Fig. 2). The lowest dose, 2 mg/kg, was rapidly absorbed. The occurrence of double or multiple peaks in the plasma drug concentrations and a prolongation of the MAT at the two higher dose levels, indicated interrupted or delayed gastric emptying, as apparent from the Wagner-

-Nelson plots. Recent experiments with duodenal administration of enprofylline appear to verify this hypothesis (Johannesson *et al.* unpublished).

The short half-life of enprofylline, 1.2-1.9 hours (Borgå *et al.* unpublished) will necessitate the use of controlled-release preparations for oral maintenance therapy. With a low rate of release of the drug, the main part of the absorption will take place in the intestine. Thus, the delayed gastric emptying observed in the present study will probably be of little clinical importance. Further studies using controlled-release preparations will have to clarify this point.

The areas under the plasma concentration-time curves (AUC^{∞}) showed a non-linear increase with increasing dose. Bioavailability calculated as $AUC^{\infty}_{oral}/AUC^{\infty}_{i.v.}$ then reached values well above 100%. At the same time recovery of unchanged enprofylline in urine declined slightly, indicating less than 100% absorption at the highest dose. Taken together the data indicate capacity limited elimination of enprofylline. In the present study this became apparent when the dose was increased from 4 to 6 mg/kg (Fig. 2, subject Nos. 2, 3 and 5, and Fig. 3). The corresponding plasma level range was from 3.5 to 6.5 mg/L. The effect was not dramatic and it will probably be of little clinical concern, since therapeutically active concentrations appear to be in the range 1 to 4 mg/L [6]. However, further studies in patients are needed to define the therapeutic plasma concentration range.

Acknowledgement: The authors would like to thank Dr Nils Johannesson, AB Draco, Lund, for valuable suggestions on the pharmacokinetic calculations.

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TABLE 2.

Maximum plasma concentrations (C_p^{max}) and mean absorption time (MAT) after oral doses of 2, 4 and 6 mg/kg enprofylline

	C_p^{max} (mg/L)			MAT (hours)		
Subject No. \ Dose mg/kg	2	4	6	2	4	6
1	2.24	3.96	——	0.3	0.8	——
2	2.26	2.89	6.42	0.4	1.3	1.4
3	2.31	3.90	5.90	0.5	0.9	1.6
5	2.20	4.00	6.37	0.7	1.5	1.9
6	1.70	2.76	4.58	0.6	0.7	0.9
7	1.66	3.91	4.46	1.0	1.7	2.2
Mean	2.06	3.57	5.55	0.58	1.15	1.60
SEM	0.12	0.24	0.43	0.10	0.17	0.22

Table 3

Area under the curve (AUC^{∞}_{oral}) and bioavailability calculated as $\frac{AUC^{\infty}_{oral}}{AUC^{\infty}_{i.v.}}$ and as $\frac{oral}{i.v.}$ 24 h urine recovery after oral doses of 2,4 and 6/mg kg enprofylline[1)]

Subject No \ Dose(mg/kg)	AUC^{∞}_{oral} (mg x h/L)			Ratio $\frac{oral}{i.v.}$ AUC^{∞} (%)			Ratio $\frac{oral}{i.v.}$ urine recovery(%)		
	2	4	6	2	4	6	2	4	6
1	6.38	14.3	-	104	117	-	108	84	-
2	7.91	17.4	30.2	89	99	114	95	98	97
3	6.87	14.9	30.4	73	79	107	101	98	93
5	6.44	14.7	32.5	93	112	157	103	103	95
6	4.79	9.40	16.9	68	67	80	104	106	85
7	7.13	18.4	29.3	82	106	112	106	104	98
Mean	6.59	14.9	27.8	85.0	96.6	114.0	102.8	98.8	93.6
SEM	0.43	1.3	2.8	5.5	8.0	12.4	1.9.	3.8	2.3

[1)] i.v. data used were obtained from experiments in the same subjects (Borgå et al unpublished)

III

Pharmacokinetics of Enprofylline in Patients with Impaired Renal Function after Intravenous Single Dose Administration

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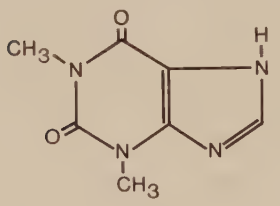
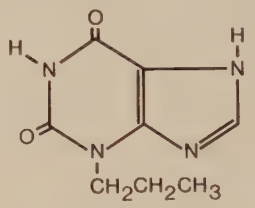
Summary: Enprofylline, a new bronchodilating drug, was given i.v. in a dose of 1.0 mg/kg to 7 healthy subjects and 14 patients with different degrees of chronic renal insufficiency. Plasma and urine concentrations of unchanged drug were followed by HPLC. In the patients plasma half-lives were prolonged and total and renal clearance reduced in direct proportion to the degree of renal insufficiency as determined by creatinine clearance. The unbound fraction of enprofylline in plasma increased from 55% in the healthy subjects to 66% in the group of patients with the highest degree of renal impairment. Volume of distribution terms, V_{β} and V_{ss}, both tended to decrease with decreasing creatinine clearance. When the volume term calculations were based on unbound drug levels in plasma, this tendency was enhanced. Side-effects were noted in 4 subjects and were to some extent related to plasma levels of the drug.

Key words: enprofylline, pharmacokinetics, renal elimination, renal insufficiency, healthy subjects, creatinine clearance

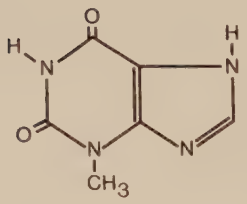
Enprofylline (p-INN, Fig. 1) is the 3-propyl derivative of xanthine. It appears to be about 5 times more potent than theophylline (Fig. 1) as a bronchodilator in both animals [16,17] and asthma patients [12]. In similarity to 3-methylxanthine [9] (3-MX, Fig. 1) which is an active metabolite of theophylline [1,8,15,21], enprofylline is mainly excreted unchanged in urine [Borgå *et al.* unpublished]. Thus the 24-h urine recovery of unchanged drug averaged 89% in 8 healthy male subjects after intravenous doses of 0.5, 1.0, and 1.5 mg/kg. In the present study we investigated the distribution and elimination of enprofylline in healthy subjects and patients with varying degrees of renal impairment. The drug was given as a single intravenous infusion of 1.0 mg/kg over 20 min.



Theophylline



Enprofylline



3-Methylxanthine

Fig. 1. Structures of theophylline, 3-methylxanthine and enprofylline .

Material and Methods

Patients

Fourteen patients, 13 males and 1 female aged 31-68 (mean 56) years with different degrees of chronic stable renal insufficiency and 7 healthy volunteers, 5 males and 2 females aged 23-37 (mean 31) years entered the study (Table 1). The patients were subdivided into two groups, I and II, according to their creatinine clearance values. Thus, the subjects fell into one of 3 equally large groups:

Group I, creatinine clearance 3-18 ml/min (n = 7)
Group II, creatinine clearance 31-74 ml/min (n = 7)
Healthy subjects, creatinine clearance 88-133 ml/min (n = 7)

None of the patients or healthy volunteers had liver disease as assessed by conventional laboratory tests. The patients with renal insufficiency were treated with one or more of the following drugs: digoxin, frusemide, β-adrenoceptor blocking agents, hydralazine and 1-α-hydroxyvitamin-D_3. The nature of the study was fully explained to the patients and volunteers. All gave their consent prior to entering the study, which was conducted with the approval of the ethical committee of the University of Linköping.

Renal function. Creatinine clearance was measured using the 24-h urine excretion of endogenous creatinine and a single plasma creatinine determination. Creatinine in plasma and urine was assayed by the Jaffé reaction with an Autoanalyzer (Technicon method M-38). Creatinine clearance values were corrected to 1.73 m^2 body surface area.

Drug administration. The subjects were allowed to follow their normal habits of eating and drinking during the investigation days. Thus coffee, tea and other xanthine containing beverages were allowed. In the morning of the first day of the study a 20 min i.v. infusion of enprofylline, 1 mg/kg (0.92-1.25 mg/kg) was given.

Table 1. Data on patients and healthy volunteers participating in the study on enprofylline pharmacokinetics in renal impairment

	Patient No.	Sex	Age	Weight (kg)	Diagnosis	Creatinine clearance (ml/min/1.73 m^2)	Serum creatinine (µmol/L)	Serum albumin (g/L)	Actual dose (mg/kg)
Severe renal impairment	1	male	31	78	chronic pyelonephritis	3	1300 before, 870 after regular hemodialysis	35	1.15
	2	"	68	70	chronic glomerulonephritis	5	1080	43	1.00
	3	"	62	84.5	nephroarteriosclerosis	8	654	48	1.25
	4	"	31	65	chronic glomerulonephritis	11	588	42	0.92
	5	"	55	65	nephroarteriosclerosis	16	285	41	1.08
	6	"	54	76	chronic interstitial nephritis	16	503	40	1.05
	7	"	66	86	nephroarteriosclerosis	18	355	35	1.05
Moderate to slight renal impairment	8	female	59	62	chronic interstitial nephritis	31	150	43	0.97
	9	male	60	78	nephroarteriosclerosis	35	180	43	1.03
	10	"	59	73.5	nephroarteriosclerosis	37	275	46	0.95
	11	"	62	71	chronic glomerulonephritis	45	160	44	0.99
	12	"	57	73	chronic glomerulonephritis	47	150	45	0.96
	13	"	64	70.5	chronic pyelonephritis	69	130	41	0.99
	14	"	58	81	nephroarteriosclerosis	74	126	42	0.99
Healthy volunteers	15	female	36	60	healthy	88	80	50	1.00
	16	male	28	89.5	"	108	80	52	1.01
	17	"	36	64	"	110	110	43	1.00
	18	female	23	70	"	114	75	51	1.00
	19	male	30	97	"	118	100	35	0.81
	20	"	37	105	"	130	105	49	1.05
	21	"	29	63	"	133	89	38	1.00

Sampling. Heparinized blood samples (5 ml) were drawn from an antecubital vein of the opposite arm using Venoject® vacuum tubes. Samples were taken immediately before and 20 and 30 min after the start of infusion and then at 5 occasions at regular intervals during the following 4 predicted half-lives. Urine was collected quantitatively in fractions. Collection times varied from 24 hours in the healthy subjects up to 60 hours in the patients with severe renal impairment. Plasma and urine samples were kept at -20°C until assayed.

Enprofylline was assayed by high performance liquid chromatography (HPLC), employing columns packed with a chemically bonded reversed-phase material of the C_{18}-type.

A mobile phase consisting of methanol/water 30/70 (v/v) containing one gram of phosphoric acid per litre was used for plasma analysis. For urine the mobile phase consisted of acetonitrile, tetrahydrofurane and 0.005 mol/L phosphoric acid buffer pH 7.5 (6/2.5/94, v/v/v).

Plasma assay. To 100 µl plasma was added an internal standard (3,7-dihydro-1-ethyl-3-(2-hydroxypropyl)-1H-purine-2,6-dione). The sample was made alkaline and then extracted for five minutes with a mixture of chloroform/isopropanol. After centrifugation the organic phase was discarded. Perchloric acid was added to the aqueous phase to precipitate residual proteins. After centrifugation the sample was injected onto the chromatographic column.

Urine assay. After the addition of internal standard (same as with plasma) to 100 µl urine, the sample was diluted ten times with either water or mobile phase before injection onto the column.

Each plasma and urine assay included calibration samples prepared in blank plasma or blank urine. In addition, low and high control samples were included in each assay for quality assessment.

The data on inter assay precision varied at different concentrations and between plasma and urine but were in no instance worse than 3.5% expressed as coefficient of variation.

Protein binding. Plasma protein binding was assayed by equilibrium dialysis [6] at room temperature (20°C) at an added enprofylline concentration of $2 \cdot 10^{-5}$ M. Plasma samples that had been kept frozen were used, since freezing and thawing appeared to have negligible effect on the binding [Tegnér et al. unpublished]. The pH of the samples was measured before the analysis and the pH of the dialysis buffer chosen so as to produce a post-dialysis value of 7.4. Minor deviations (± 0.1 pH units) in the post-dialysis pH could nevertheless occur. The per cent binding was then corrected for this deviation using the known linear relationship between binding and pH [Tegnér et al. unpublished].

Pharmacokinetic calculations. Area under plasma concentration-time curves (AUC) were determined by the trapezoidal rule. AUC from the last plasma concentration, C_n, to infinity was estimated as C_n/β. Beta was estimated as the slope of the apparently linear last portion of the ln c_p vs time curves, $t_{\frac{1}{2}}$ as ln $2/\beta$. Beta and $t_{\frac{1}{2}}$ were also estimated from the excretion rate vs time curves. Total plasma clearance, CL was estimated as Dose/AUC, and volume of distribution V_{area} or V_β during the beta elimination phase as CL/β. Volume of distribution steady-state V_{ss}, was calculated as follows [3,20]:

$$V_{ss} = \frac{\text{Dose} \cdot \text{AUMC}_\infty}{[\text{AUC}_\infty]^2} - \frac{\text{Dose} \cdot \text{Infusion time}}{2 \cdot \text{AUC}_\infty}$$

Here AUMC_∞ is the area under the first moment of the plasma curve, i.e., $\int_0^\infty t \cdot c_p dt$. This area was measured as the trapezoidal area plus the extrapolated area which in this case is $\frac{C_n}{\beta^2} + \frac{C_n \cdot t_n}{\beta}$.

Comparison of the above calculation procedure were performed with procedures assuming exponential rather than linear decay of the plasma curve between two consecutive time points. Using trapezoidal areas V_{β} was generally underestimated by 1-2% while V_{ss} was generally underestimated by 3-5%, deviations that were considered to be negligible.

Distribution volumes based on unbound drug concentrations in plasma, V^u_{β} and V^u_{ss}, were calculated as V_{β}/f_u and V_{ss}/f_u respectively. The unbound fraction in plasma, f_u, is virtually constant at the low concentrations obtained after a dose of 1 mg/kg.

Renal clearance, CL_r, was estimated by dividing the total recovery, X_n of enprofylline in urine up to a certain time, t_n (usually the last measurable point on the plasma concentration curve) by the corresponding AUC. Thus $CL_r = \frac{X_n}{\int_0^{tn} c_p \, dt}$

The non-renal clearance was calculated as $CL - CL_r$. Pharmacokinetic calculations were facilitated by use of a computer program run on a desk-computer Hewlett-Packard 9835A.

Statistics

The results are given as mean ± SD. The significance of correlation coefficients (and of mean differences between the two groups of patients with renal insufficiency and healthy volunteers) was assessed by t-tests for independent samples. Results corresponding to probabilities less than 0.05 in a two tailed test were considered statistically significant.

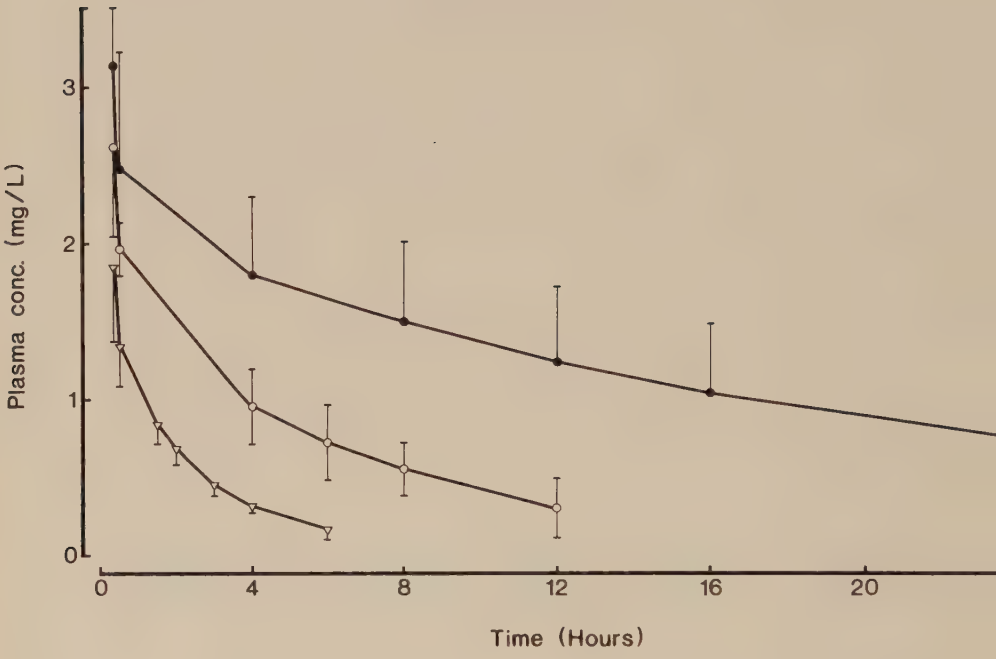


Fig. 2. Mean plasma levels ± SD of enprofylline plasma concentrations in patient group I ●—●, patient group II ○—○, and healthy subjects ▽—▽.

Results

The mean plasma concentrations of enprofylline were higher immediately after termination of the infusion in patients with severe renal impairment (creatinine clearance 3-18 ml/min; patient group I) compared to patients with moderate to slight degree of renal impairment (creatinine clearance 31-74 ml/min; patient group II) and healthy volunteers (Fig. 2). The mean plasma concentration in group I 4 hours after dose was about 6 times and in group II 3 times as high as in the healthy volunteers. In two patients belonging to group I (creatinine clearances 3 and 16 ml/min) the plasma concentrations were 0.42 and 0.30 mg/L, still 60 hours after dose, whereas in healthy subjects enprofylline plasma levels were below the lower limit of determination, 0.1 mg/L, 8 hours after dose.

In the healthy volunteers the plasma half-life of enprofylline ranged between 1.5-2.6 (mean 1.8) hours, in group II patients between 2.7-7.2 hours and in group III patients between 8.6-26.6 hours.

Plasma half-lives were slightly shorter than half-lives calculated from excretion rates of unchanged enprofylline in urine. The following equation was obtained by linear regression analysis:

$$(T_{\frac{1}{2}})_{plasma} = 0.92(T_{\frac{1}{2}})_{urine} + 0.16 \quad (\text{unit} = \text{hours}, r = 0.98, p < 0.001)$$

Values of 24 h urine excretion of unchanged drug expressed as per cent of given dose are presented in Table 2.

Enprofylline renal clearance varied from 2.1 to 357 mL/min and was strongly related to creatinine clearance ($r = 0.94$, $p < 0.001$, Fig. 3b, Table 2). A similar relationship to creatinine clearance was seen with the elimination rate constant, β, of enprofylline ($r = 0.92$, $p < 0.001$, Fig. 3a). Non-renal clearance was lower in patients with severe renal insufficien-

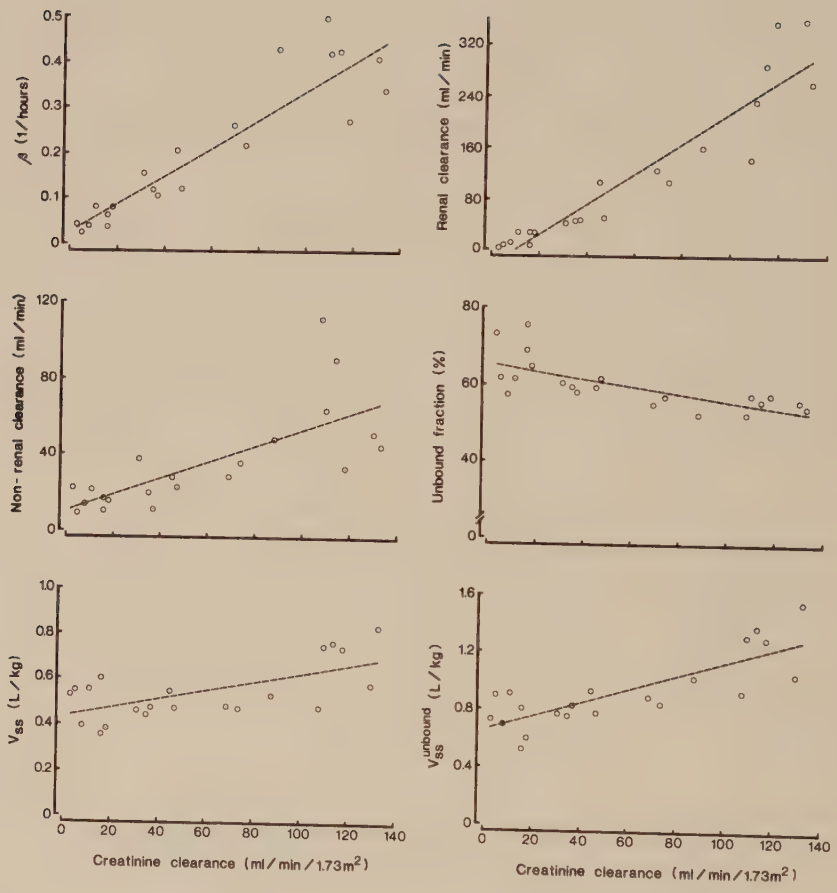


Fig. 3. Relationship between creatinine clearance and a) the elimination rate constant, β , b) renal clearance, c) non-renal clearance, d) unbound fraction in plasma, e) V_{ss} , d) V^{u}_{ss} of enprofylline in 7 healthy subjects and in 14 patients with impaired renal function.

cy than in healthy subjects (Table 2) and this parameter was too significantly related to creatinine clearance ($r = 0.74$, $p < 0.001$, Fig. 3c). However, there was also a similar decrease in non-renal clearance with increasing age ($r = -0.67$, $p < 0.001$).

The plasma protein binding averaged 45.0% (42.7-47.7) in healthy subjects and decreased with decreasing renal function to 41.4% (38.5-45.1) and 33.9% (24.6-42.7) in patient groups II and I, respectively. Values of unbound fraction are presented in Table 2. There was a significant inverse linear relationship between creatinine clearance and the unbound fraction of enprofylline ($r = -0.69$, $p < 0.001$, Fig. 3d). The values of plasma albumin were slightly lower in the two patient groups (mean values 43.4 and 40.6 g/L in groups II and I, respectively) compared with the healthy subjects (mean value 45.4 g/L). Recalculation of the figures of unbound fraction to a "normal value" of 45 g/L of albumin was performed [14]. Recalculated values (Table 2) showed a similar correlation with creatinine clearance ($r = -0.67$, $p < 0.001$) indicating that the relationship could not be explained by lower albumin in patients with renal impairment.

V_β values of volume of distribution were virtually linearly related to values of V_{ss}, volume of distribution at steady state ($V_\beta = 1.076\ V_{ss} + 0.0086$, unit = L/kg, $r = 0.994$, $p < 0.001$). V_β and V_{ss} (Fig. 3e) were both significantly related to creatinine clearance ($r = 0.68$, and 0.67 respectively), lower values being obtained in subjects with impaired renal function.

Both volume terms were also calculated from unbound drug levels to eliminate the influence of variability in plasma protein binding. The corresponding parameters V^u_β and V^u_{ss} (Fig. 2f) were again significantly related to creatinine clearance ($r = 0.82$ and 0.82 respectively).

Table 2. Pharmacokinetic parameters in patients with renal impairment and in healthy volunteers after 20 min intravenous infusion of enprofylline 1.0 mg/kg body weight

Patient No.	Creatinine clearance (ml/min/1.73 m^2)	$t_{½}$ plasma (hours)	Unbound fraction (%)	Unbound fraction corrected[1] (%)	V_{ss} (L/kg)	Total plasma clearance (ml/min)	Renal clearance (ml/min)	Non-renal clearance (ml/min)	24 h urine excretion (% of given dose)
1	3	18.5	73.1	67.9	0.53	25	2.1	23	4.7
2	5	26.6	61.7	60.6	0.55	16	7.0	9.2	18.6
3	8	17.7	57.3	58.8	0.40	24	10	14	29.9
4	11	8.8	61.3	59.7	0.55	49	27	22	46.2
5	16	11.6	75.4	73.1	0.60	44	27	17	19.3
6	16	18.0	68.9	66.9	0.36	17	7	10	51.3
7	18	8.6	64.7	58.8	0.39	42	26	16	48.0
8	31	4.5	60.3	59.2	0.46	79	41	38	50.8
9	35	5.7	59.3	58.2	0.44	65	45	20	59.8
10	37	7.2	57.9	58.5	0.48	58	47	11	67.6
11	45	3.6	59.2	58.6	0.55	134	106	28	77.5
12	47	6.0	61.5	61.5	0.47	73	50	23	65.9
13	69	2.7	54.9	52.6	0.48	153	125	28	79.9
14	74	3.4	56.8	55.1	0.47	143	107	36	74.6
15	88	1.7	52.3	54.9	0.53	208	160	48	78.4
16	108	1.5	52.3	55.9	0.48	254	143	111	78.8
17	110	1.7	57.3	56.2	0.75	296	232	64	78.1
18	114	1.7	55.7	58.8	0.76	378	288	90	76.2
19	118	2.6	57.3	51.0	0.74	387	353	34	93.2
20	130	1.7	55.7	57.9	0.58	408	357	51	86.6
21	133	2.0	54.1	49.9	0.83	305	260	45	84.3

[1] Corrected (recalculated) value corresponds to an albumin level of 45 g/L

Adverse effects occurred in 3 patients with severe renal failure at 1-1.5 h after start of infusion with a duration of 5-12 h and included headache (pat. No. 1) nausea and vomiting (pat. Nos. 1, 3, and 4), and in one healthy volunteer (No. 16) headache from 0.5 to 12 h after dose.

Discussion

In studies in rats using ^{14}C-labelled enprofylline approximately 98% of an i.v. dose was recovered as unchanged drug in the urine [Tegnér et al. unpublished]. In the present study the main portion, 82 ± 6% (mean ± SD) of a single i.v. dose was recovered in the 24 h urine of the healthy subjects. This is similar to the 89 ± 4% (mean ± SD) found in a previous study in 8 healthy male subjects who received 0.5, 1.0 and 1.5 mg/kg enprofylline i.v. [Borgå et al. unpublished]. The less than 100% recovery in urine of healthy subjects during a total sampling time exceeding 10 half-lives suggests the existence of other elimination routes. This is corroborated by the patient data, especially in the group I patients where non-renal clearance (16 ± 5 ml/min, mean ± SD) was of the same order of magnitude as the renal clearance (15 ± 11 ml/min). In the group II patients the average non-renal clearance still accounted for approximately 1/4 of the total clearance. However, quite in contrast to what one would conceive from the above results, the non-renal clearance in absolute figures decreased dramatically with decreasing renal function (Fig. 3c). The reason for this is unknown, but it is possible that it, at least to some extent, may be explained by differences in age, since there was also a significant inverse relationship between age and non-renal clearance of enprofylline. Both increased and decreased rate of metabolism has previously been associated with uremia. Thus the half-life of antipyrine is reduced in uremic subjects [13] while the metabolic clearance of cimetidine, which is main-

ly metabolized by sulphoxidation is reduced in uremic patients [11]. The metabolic pathways of enprofylline have so far not been identified. This makes any further speculation impossible on the reason for the observed decrease in non-renal clearance of enprofylline in patients with impaired renal function.

As expected, the renal clearance of enprofylline decreased in relation to decreasing creatinine clearance. It should be noted, however, that there was a large variation in renal clearance of enprofylline in healthy subjects (143-357 mL/min) that was not possible to relate to a similar variability in creatinine clearance (total range 88--130 mL/min). This does not mean that a relationship between the two parameters does not exist in kidney-healthy subjects since we studied only 7 subjects. This issue would be desirable to address in a much larger population.

As to the mechanisms of renal elimination of enprofylline some conclusions can be made. Since about 45 % of enprofylline is bound to plasma proteins, its glomerular filtration rate in healthy subjects should approximate 50-70 mL/min. The high renal clearance of enprofylline in this category in the present (143-357 mL/min) and a previous study (200--407 mL/min) [Borgå et al. unpublished] suggests that the drug is excreted predominantly by active tubular secretion. Active tubular secretion has been described with many drugs which have in common with enprofylline that they are weak acids. In many cases probenecid has been effective to reduce their renal clearance. In fact we have observed a considerable prolongation in enprofylline half-life in healthy volunteers after pretreatment with probenecid (unpublished observations).

As previously noted [Tegnér et. al. unpublished], the plasma protein binding of enprofylline was relatively low, about 45%, in healthy subjects. The main binding protein of enprofylline is probably albumin [Tegnér et al. unpublished], in similarity with findings with theophylline [5].

Assuming binding only to albumin, we corrected all binding figures for the interindividual differences in plasma albumin levels. Original and recalculated values of unbound fraction of enprofylline showed the same inverse correlation with creatinine clearance. Similar findings have been done with several other drugs bound to plasma albumin, e.g. fenytoin [14]. Thus decreased albumin levels can contribute to, but not explain, the decreased drug binding in uremia [4]. Decreased binding in uremia has been observed with many drugs that have in common with enprofylline that they are weak acids (i.e. anionic at physiological pH) and that they are bound to plasma albumin [4,18]. Binding inhibitors present in uremic plasma, and bound to plasma albumin, is the most probable explanation to this phenomenon [19].

Generally, the apparent volume of distribution of a drug tends to increase when plasma binding is decreased [7,10]. In most cases this difference is entirely due to the use of total drug levels when the volume term is calculated. When the calculation of the latter is based upon unbound drug levels, this difference is generally eliminated, which thus suggests that tissue binding is unchanged. With enprofylline we found a <u>decreased</u> volume of distribution (V_{β} or V_{ss}) in the patients, which was even more pronounced when these parameters were calculated from unbound drug levels (V^{u}_{β}, V^{u}_{ss}). The reduction was significantly correlated to creatinine clearance (cf. Fig. 3f). It should be noted, however, that the V_{ss} values in healthy subjects in the present study (0.67 ± 0.13, mean ± SD) were higher than those found in a previous study (0.51 ± 0.06, mean ± SD) [Borgå <u>et al.</u> unpublished]. The factors controlling the volume terms are not well understood. The differences between the present and the previous study may therefore be due to differences in the composition of the relatively small groups of healthy subjects studied. Another factor to consider is age. Our healthy subjects were younger (mean

age 31) than our patients (mean age 56). A reduction in volume of distribution of unbound theophylline has been demonstrated in elderly subjects, 70-85 years of age [2]. Since distribution parameters of enprofylline and theophylline, i.e. protein binding and volume terms, are very similar, there may exist a similar age dependency for the volume of distribution of enprofylline as found with theophylline [2].

The occurence of side-effects in the present study were to some extent related to the plasma levels of enprofylline. High plasma levels were noted in patient group I already after finished 20 min i.v. infusion (Fig. 2). Adverse effects of enprofylline occurred in 3 of these patients (Nos. 1, 3 and 4) but in only one healthy subject. The latter had higher plasma concentrations at 20 min (2.4 mg/L) and at 30 min (1.75 mg/L) than the rest of the group which had 1.29-2.38 and 1.00-1.59 mg/L, respectively at these time-points. However, there were no apparent differences in plasma concentrations of enprofylline between group I patients with and without side-effects.

In therapy it seems reasonable to reduce the i.v. dose of enprofylline in patients with impaired renal function to avoid side-effects. A plasma level above 1 mg/L has been shown to be associated with a significant increase in forced exspiratory volume in asthmatic patients [12]. Plasma levels above 1 mg/L throughout the dosage interval would be obtained using the following preliminary dosage scheme (i.v. administration):

0.75 mg/kg every 12 h in patients with creatinine clearance <20 ml/min or serum creatinine >300 µmole/L.

1.0 mg/kg every 6 h in patients with creatinine clearance 30-50 ml/min or 150 µmole/L < serum creatinine ≤300 µmole/L

1.0 mg/kg every 4 h in patients with creatinine clearance 50-75 ml/min or 115 µmole/L ≤ serum creatinine ≤150 µmole/L.

However, more experience of the pharmacokinetics and effects of enprofylline after single and during repeated oral and intravenous dosing is needed both in subjects with normal renal function and in subjects with renal impairment to form a basis for definite dosage recommendations.

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IV

Tolerance and some Circulatory Effects of Intravenous and Oral Enprofylline in Healthy Volunteers

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Abstract. Enprofylline (3-propylxanthine), a novel bronchodilating xanthine derivative that seems to lack adenosine antagonistic potency was given intravenously to eight recumbent healthy male volunteers in the doses 0.5, 1 and 1.5 mg/kg body-weight (i.v.) and to six of them also orally in the doses 2, 4 and 6 mg/kg. Mean enprofylline plasma levels ranged between 1.6 and 4.4 mg/L (8.2-22.2 µmol/L) after i.v., and between 1.9 and 5.5 mg/L (9.8-27.9 µmol/L) after oral administration. Enprofylline was rapidly and completely absorbed and had an elimination half-life of approximately 2 h. About 90% of the dose given by either route was recovered as unchanged drug in the urine. A slight but significant increase in heart rate was seen at peak plasma levels after each of the highest i.v. and oral doses. At these dose levels the heart rate response to orthostatic tests was significantly increased by enprofylline. Adverse reactions were mild and short-lasting and occurred most frequently after the two highest i.v. and oral doses. Headache and nausea were noted in 5 of the 24 i.v. experiments and in 9 of the 17 times that enprofylline was given orally. In conclusion, the circulatory effects of enprofylline were small and the adverse reactions mild. Further clinical studies with enprofylline seem warranted.

Key words: Enprofylline - plasma concentration - healthy volunteers - adverse reactions - circulatory effects

Accumulated experience with theophylline has made possible a more effective treatment of asthma, but also highlighted some clinical disadvantages with this xanthine derivative. About 90 % of a given dose of theophylline is eliminated by metabolism, the rate of which can vary greatly between and within individuals (Jenne et al. 1972). Toxic theophylline levels may be produced due to unpredictable, long half-lives or due to the fact that theophylline is often given i.v. to patients who are on oral maintenance therapy with slow-release theophylline preparations. A xanthine bronchodilator less prone to produce extra-pulmonary effects, less dependent on metabolizing capacity, and eliminated mainly by the renal route could be an interesting alternative to theophylline. Enprofylline, INN, (3-propylxanthine) may fulfil these requirements. It seems to lack a number of theophylline-like effects, e.g., CNS-stimulant behavioural and diuretic effects (Persson 1982, Persson et al. 1982). Such pharmacological differences may be due to the poor ability of enprofylline and potent ability of theophylline to antagonize cellular actions of adenosine. Furthermore, enprofylline has been shown in rats to be well absorbed and to be eliminated almost quantitatively by renal excretion of unchanged drug (Persson et al. 1982). Besides being a drug candidate, enprofylline can serve as a scientific tool since pharmacodynamic dissimilarities between this xanthine and theophylline may show the physiological importance of adenosine and its antagonism (Persson 1982).

In the present study, different intravenous (i.v.) and oral doses of enprofylline were given to healthy male volunteers. Plasma concentrations of enprofylline and urinary recovery of unchanged drug were determined. Adverse reactions, and heart rate and blood pressures during rest and in orthostatic tests were recorded. Since this was the first time enprofylline was given to man, the experiments were performed as an open study.

METHODS

Subjects

Eight healthy male subjects volunteered to participate in the i.v. study. Six of them were also given oral enprofylline. Mean age was 26.7 years (range 23 - 34 years) and mean weight 75.6 kg (range 64 - 94 kg). Physical examination and haematological, biochemical, and electrocardiographic indices were normal in all. None was taking any prescription or non-prescription drug. They were either medical students or members of the scientific staff of the Department of Clinical Pharmacology. Each subject received extensive information on relevant pharmacological data and on the trial design. The study protocol was approved by the Ethical Committee of the University of Lund and all subjects gave written consent. One subject did not receive the 6 mg/kg dose. He refrained from the study for personal reasons, not because of side effects.

Procedure

To fulfil optimal safety requirements, the investigations were non-blind and performed at the Intensive Care Unit of the Department of Medicine, University Hospital of Lund. The same physician was present throughout each day of the study. The subjects were given single increasing doses of enprofylline i.v. and orally at six different occasions each separated by two weeks.

The dose levels chosen were based on the potency ratio of 1:3 to 1:6 between theophylline and enprofylline in animal studies for many effects, including bronchodilation. They had also been tested in pilot studies preceding the present investigation. In the pilot studies signs of vasodilatation (short-lasting facial flush) had been observed which motivated the performance of orthostatic tests in the present study. They also pointed at a story of migraine being an exclusion criterion for enprofylline treatment, since one such subject had an attack of migraine after receiving the drug. No alcohol, coffee, tea or cola drinks were permitted for the 24 hours preceding admission or during the days of investigation. A light breakfast was permitted not later than 1 hour before admission. The subjects were observed for 10 hours. They were allowed to sleep at home in the evening, but blood samples were drawn regularly and urine was collected during 24 hours after each dose. Haematological and biochemical tests were repeated 2, 7 and 14 days after each dose. Solutions of enprofylline (provided by AB Draco, Lund) contained 10 mg/ml (for injection) and 1.32 mg/ml (for oral ingestion).

The subjects arrived at the unit at 07.30 in the morning and an indwelling venous cannula was established. After rest for 1 h, basal values (before drug) were recorded in recumbent position and at orthostatic tests. At 08.30 an intravenous infusion of 0.5, 1.0 and 1.5 mg/kg body-weight, respectively, was given during 10 min. Electrocardiogram (ECG) was monitored continuously. Heart rate (HR) and blood pressure (BP) with a manometer were recorded regularly for 120 min, always by

the same investigator. The subjects remained supine during the first 4 hours, except for orthostatic tests (heart rate and blood pressure were measured after 2 and 4 min of quiet standing). The subjects were questioned about adverse reactions according to a protocol including nausea, headache, palpitations, tremor, restlessness, loss of appetite and gastrointestinal disturbance.

On the days of the oral study single doses of 2 mg/kg, 4 mg/kg and 6 mg/kg of enprofylline in a water solution (450 ml) were given to six of the volunteers at 08.30 and the same recordings as in the i.v. study were made for 600 min except for the orthostatic tests which were of 2 min duration. Three subjects had a small breakfast more than 1½ h before the intake of the drug, the others were fasting. Venous blood samples were drawn regularly and urine was collected during 24 hours.

Drug analyses

Blood samples for determination of enprofylline were collected in heparinized tubes (Venoject^{R}) and centrifuged within 10 min. The samples of plasma and urine were frozen within one hour and stored at $-20\ ^{o}C$ until analyzed.

Enprofylline in plasma and urine was determined by liquid column chromatography. A detailed description of the analytical procedures is given elsewhere (Edholm & Heintz, in preparation). The method is highly specific for enprofylline, the sensitivity limit 0.1 mg/L and the interassay variation expressed as the coefficient of variation less than 3.5 % at 0.4 and 3.0 mg/L.

Statistical methods

The circulatory values post drug were compared to the mean of two measurements made after 1 h of rest before drug administration. The significance of differences between pre-treatment and treatment values were evaluated by Student's paired *t*-test. Results corresponding to probabilities less than 0.05 were considered statistically significant. Mean values are given ± SEM.

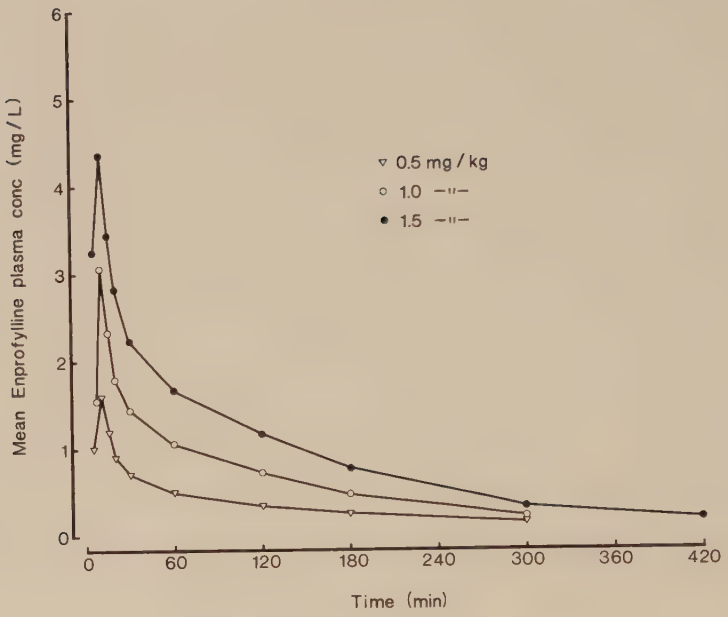

Fig. 1. Mean plasma concentrations of enprofylline in eight healthy volunteers after 10 min i. v. infusion at three different dose levels.
S. I. conversion: μmole/L = mg/L · 5.1.

RESULTS

Plasma concentrations and urinary recovery

Mean plasma concentrations of enprofylline after i.v. administration are shown in Fig.1. At the end of infusion of 0.5, 1.0 and 1.5 mg/kg body-weight the plasma concentrations amounted to 1.60 ± 0.13 mg/L (8.2 ± 0.7 µmol/L), 3.07 ± 0.28 mg/L (15.7 ± 1.4 µmol/L) and 4.36 ± 0.49 mg/L (22.2 ± 2.5 µmol/L), respectively. The decline of the plasma concentrations indicated a plasma half-life for enprofylline of about 2 h. The recovery of unchanged drug in urine sampled for 24 h after infusion amounted to 89 ± 4 %.

Mean maximum plasma concentrations after oral intake of enprofylline were 1.92 ± 0.16 mg/L (9.8 ± 0.8 µmol/L), 3.69 ± 0.22 mg/L (18.8 ± 1.1 µmol/L) and 5.48 ± 0.4 mg/L (27.9 ± 2.1 µmol/L) after 2, 4 and 6 mg/kg, respectively (Fig. 2). Maximum plasma concentrations were attained within 2 h and the drug seemed to be almost completely absorbed. Thus, the recovery of unchanged drug in urine sampled for 24 h amounted to 88 ± 2 % of the given dose.

Heart rate and blood pressure

Supine position. When the subjects were given i.v. or oral enprofylline in the supine position, a statistically significant increase in heart rate was recorded at the time of the highest plasma concentrations, i.e., at the completion of the 1.5 mg/kg infusion, and 30 - 240 min after oral intake of 6 mg/kg (Table 1). No siginificant influence on blood pressure was recorded at any enprofylline dose level compared to before drug, neither after i.v. infusion nor after oral intake.

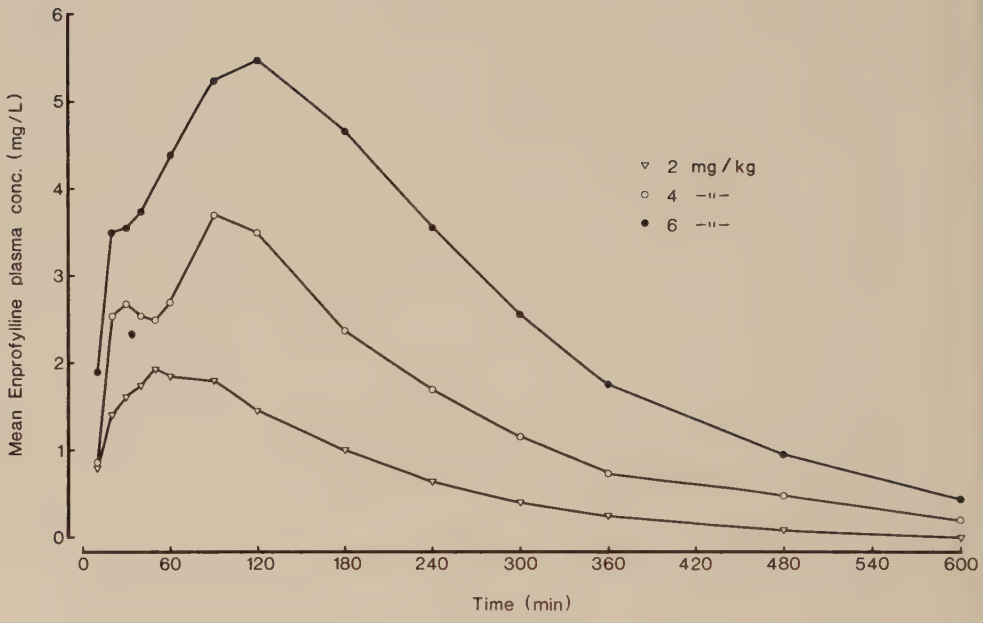

Fig. 2. Mean plasma concentrations of enprofylline in six healthy volunteers after oral administration at three different dose levels. S. I. conversion: µmole/L = mg/L · 5.1.

Orthostatic tests. Occasional vasovagal reactions with bradycardia, fall in blood pressure and faintness occurred both before and after drug administration in 3 out of the subjects (i.v.). Heart rate and blood pressure changes at these instances were not included in the calculation of the mean values for control and drug responses.

Enprofylline augmented significantly the orthostatic heart rate response only after the highest i.v. dose (1.5 mg/kg) and after 4 and 6 mg/kg orally (Table 2). A drug-induced decrease in systolic blood pressure was observed immediatly after the end of infusion of 1.5 mg/kg and 60 - 120 min after oral intake of 4 and 6 mg/kg (Table 3). A significantly decreased diastolic blood pressure response was recorded immediately after 10 min infusion of 1.5 mg/kg (Table 4). The heart rate and blood pressure responses were of the same magnitude after 2 and 4 min standing. No changes in ECG were observed during the experiments.

Tolerance

The incidence of adverse reactions is accounted for in Table 5. After i.v. administration nausea and headache occurred. Both effects had a duration of less than ½ h. A facial flush lasting for less than 3 - 4 min was noted by two subjects immediately after infusion of the two larger doses of enprofylline. After i.v. infusion 8 out of 24 administrations were associated with some adverse reaction.

TABLE 1.

Heart rates (beats/min) in supine position in healthy volunteers before and after various doses of i.v. and oral enprofylline.

	Intravenous (n = 8)			Oral (n = 6)		
	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg (n=5)
Before drug	63.9 ± 1.7	58.3 ± 2.4	59.4 ± 3.3	57.4 ± 2.5	55.3 ± 1.3	54.5 ± 1.6
10 min	64.3 ± 1.3	63.1 ± 4.0	67.0 ± 4.4 **	–	–	–
30 min	61.5 ± 2.4	61.9 ± 5.4	60.6 ± 2.9	62.7 ± 1.8	59.2 ± 1.0	63.0 ± 2.3 **
60 min	59.8 ± 2.2	57.3 ± 2.2	56.8 ± 3.2	62.3 ± 1.2	60.0 ± 1.3	61.0 ± 2.2 *
120 min	58.0 ± 2.4	60.3 ± 3.0	59.6 ± 3.3	60.0 ± 1.0	59.8 ± 2.9	63.0 ± 2.6 *
180 min	–	–	–	64.0 ± 2.6	59.7 ± 2.1	72.4 ± 5.0 *
240 min				64.0 ± 2.9	61.0 ± 2.9	71.2 ± 4.6 *

** $p < 0.01$

* $p < 0.05$

Oral administration of the drug also produced nausea and headache (Table 5). These effects were not clearly dose--dependent in individual subjects. Generally, the nausea had a duration of about 1 h and occurred just post maximum plasma levels, while the headache was most frequently noted 3 - 5 h after administrations. The headache was of the pulsating type and lasted from about 1 h to 4 - 5 h.

The feeling of facial flush in one subject after oral administration of 4 mg/kg enprofylline had a duration of about 1 h. The epigastric pain in one subject occurred 20 min after the drug ingestion and lasted for about 1 h. Nine out of 17 oral administrations were associated with some adverse reaction.

The adverse effects associated with i.v. and oral enprofylline were mild. Most subjects took a nap during the recumbent part of the experiment and no sign of CNS-stimulant behavioural effects (restlessness, tremor etc) could be detected.

TABLE 2.

Heart rate increase (beats/min) in orthostatic tests (after 2 min upright position) in healthy volunteers before and after various doses of i.v. and oral enprofylline.

	Intravenous (n = 8)			Oral (n = 6)		
	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg (n = 6)
Before drug	19.5 ± 2.8	19.3 ± 3.5	18.6 ± 2.8	19.6 ± 2.6	13.3 ± 2.1	19.4 ± 3.9
10 min	22.0 ± 5.4	23.4 ± 4.2	30.6 ± 3.8**	–	–	–
30 min	24.0 ± 1.8	20.8 ± 6.9	29.9 ± 5.8*	18.3 ± 5.3	24.3 ± 4.5	28.4 ± 4.7
60 min	19.8 ± 2.0	19.1 ± 4.3	29.4 ± 4.3*	21.8 ± 5.6	35.2 ± 5.1*	35.6 ± 3.1**
120 min	20.6 ± 2.1	18.8 ± 2.6	23.9 ± 4.1	22.0 ± 3.9	32.5 ± 6.1*	36.0 ± 4.4*

** $p < 0.01$

* $p < 0.05$

DISCUSSION

Results from experiments on rats showing that enprofylline was well absorbed and eliminated mainly by the kidneys as unchanged drug (Persson et al. 1982) were confirmed in this study on human volunteers. When given orally in doses ranging from 2 to 6 mg/kg, dose-related peaks in plasma concentration were generally obtained within 2 h, and absorption was almost complete as judged from urinary recovery data. The plasma concentrations obtained on both oral and i.v. administration could be expected to be clinically relevant if the potency ratio relative to theophylline (5:1) for production of bronchodilatation in vitro (Persson et al. 1981) were valid also in man. Preliminary data in patients with chronic obstructive lung disease have supported such an assumption (Lunell et al. 1982). Enprofylline seemed not to be metabolized to any significant degree and was excreted almost unchanged in the urine. This should make enprofylline insensitive to variations in the metabolizing capacity of the liver, which is a main source of the wide range of half-lives found with theophylline. It is further conceivable that extreme half-lives of the drug could be predicted by tests of renal function. The short half-life of enprofylline in subjects with normal renal function should be advantageous when rapid adjustments of plasma levels are desired, as may be the case in i.v. treatment of severe acute asthma. On the other hand, it would make sustained release formulations necessary for oral maintenance treatment.

TABLE 3.

Systolic blood pressure decrease (mm Hg) in orthostatic tests (after 2 minutes in upright position) in healthy volunteers before and after various doses of i.v. and oral enprofylline.

	Intravenous (n = 8)			Oral (n = 6)		
	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg (n = 5)
Before drug	8.4 ± 3.4	1.3 ± 3.2	3.4 ± 3.2	4.2 ± 2.4	0.8 ± 1.8	0.5 ± 0.5
10 min	6.2 ± 6.3	5.6 ± 4.6	23.8 ± 7.2*	–	–	–
30 min	5.6 ± 2.6	8.1 ± 6.0	10.0 ± 8.0	5.8 ± 3.0	10.0 ± 5.0	5.0 ± 7.1
60 min	1.9 ± 3.3	0.6 ± 4.2†	4.4 ± 3.2	2.5 ± 3.3	6.7 ± 2.8*	3.0 ± 2.0
120 min	2.5 ± 3.6	5.6 ± 4.0	4.3 ± 4.0	0.8 ± 1.5	15.0 ± 5.8*	17.0 ± 4.9*

† Increase

* $p < 0.05$

The effects of enprofylline on heart rate and blood pressures were small both when the drug was given i.v. and orally. Positive chronotropic and vasodilator actions could be predicted from experiments on animals (Persson et al. 1983) and were also found in the present study. In the supine position the highest doses produced a moderate (< 10 beats/min) increase in heart rate which could be roughly related to the maximum plasma concentrations. Effects on blood pressure, although small, were found in the orthostatic tests. The normal cardiovascular adaptation to standing consists of an increase in heart rate, a decrease in systolic blood pressure and a rise in diastolic pressure. These changes are relatively consistent after 2 min of standing (Drischel et al. 1963, de Marées 1976). The normal pattern of orthostatic response was retained also after enprofylline although the highest i.v. dose and the two highest oral doses tended to augment the heart rate increase and systolic blood pressure decrease. The only effect on diastolic pressure attributable to the drug was a short-lasting decrease immediately after infusion of 1.5 mg/kg, possibly reflecting that the vasodilator properties of enprofylline overcame the normal reflex-induced vasoconstriction.

The 0.5 mg/kg i.v. dose was not associated with any adverse effects, but in the higher doses enprofylline produced headache, nausea and facial flush. These adverse reactions were, thus, similar to those reported after the adenosine blocking xanthines caffeine and theophylline (cf. Andersson and Persson 1980). Because of the limited number of subjects the present study does not allow a quantitative conclusion about the frequency

TABLE 4.

Diastolic blood pressure increase (mm Hg) in orthostatic tests (after 2 minutes in upright position) in healthy volunteers before and after various doses of i.v. and oral enprofylline.

	Intravenous (n = 8)			Oral (n = 6)		
	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg (n=5)
Before drug	5.3 ± 1.6	7.2 ± 2.5	10.0±2.1	6.3 ± 1.4	7.5 ± 0.9	8.8 ± 1.2
10 min	2.5 ± 3.8	5.0 ± 4.6	0.6±4.8*†	–	–	–
30 min	7.5 ± 3.0	8.1 ± 4.9	8.1 ± 5.7	5.8 ± 3.3	0.8 ± 4.5	6.0 ± 4.3
60 min	8.8 ± 1.3	13.1 ± 2.3*	9.4 ± 2.9	10.8±2.7	6.6 ± 3.1	4.0 ± 6.6
120 min	8.6 ± 3.2	8.7 ± 2.1	8.1 ± 2.8	4.2± 2.7	1.7 ± 5.6	10.0± 4.5

* $p < 0.05$

† decrease

and severity of these reactions. In cats and dogs enprofylline was slightly more potent than theophylline to produce nausea, and it was suggested that this effect could be a CNS-action unrelated to adenosine antagonism (Persson & Erjefält 1982). The present finding that the poor adenosine antagonist enprofylline produced headache suggests that also this action may be unrelated to adenosine antagonism. The headache was of a pulsating type making it likely that a contributing factor could be the vasodilator action of enprofylline. Both nausea and headache may be xanthine-induced effects that are pronounced in drug-naive subjects as in the present panel and that may significantly subside by continuous treatment (cf. Rall 1980). The adverse reactions produced by enprofylline in this first study in man cannot be considered as a hindrance for further evaluation of the compound as a drug candidate and as a clinical pharmacological tool for elucidation of the importance of adenosine dependent mechanisms.

Table 5

No. of adverse reactions after i.v. and oral enprofylline in healthy volunteers.

	Dose mg/kg	Nausea	Headache	Flush	Other
i.v.	0.5	-	-	-	-
	1.0	1	2	2	-
	1.5	3*	-	2	-
oral	2	1	1	-	-
	4	4	2	1	-
	6	4	2	-	1 (epigastric pain)

* 2 subjects vomited

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Eur J Clin Pharmacol (1982) 22: 395–402

European Journal of
Clinical Pharmacology
© Springer-Verlag 1982

Effects of Enprofylline, a Xanthine Lacking Adenosine Receptor Antagonism, in Patients with Chronic Obstructive Lung Disease

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Summary. Enprofylline, a xanthine-derivative shown experimentally to lack universal adenosine receptor antagonism, has been examined in patients with partly reversible, chronic, obstructive lung disease. Significant bronchodilation was produced by enprofylline 2 mg/kg, giving a peak plasma concentration of 3.0 ± 0.6 μg/ml (mean ± SD). A dose of 2 + 4 mg/kg dilated the bronchi at least to the same extent as theophylline 9.2 ± 0.9 mg/kg (plasma level 18.5 ± 4.7 μg/ml). Neither at the low nor at the high dosage (2 + 4 mg/kg), giving plasma concentrations of 8.5 ± 1.4 μg/ml, did enprofylline produce theophylline-like CNS effects, such as restlessness and tremor, but it did exhibit some of the innocuous side effects expected with xanthine derivatives, such as epigastric discomfort and headache. The comparison with theophylline was limited because different dosage forms had to be used (solution and tablets), which for example, resulted in different absorption rates. Nevertheless, the present findings indicate enprofylline to be a potent bronchodilator in patients with obstructive lung disease, suggesting that adenosine-receptor antagonism is not involved in the bronchodilator effects of xanthines.

Key words: enprofylline, theophylline, obstructive lung disease; adenosine, antagonism, bronchodilatation, plasma levels

Elucidation of the clinical efficacy and of the pharmacokinetics of theophylline, together with improved possibilities for monitoring its plasma concentration have led to a reappraisal of theophylline in asthma therapy (Jenne et al. 1972; Ellis et al. 1976; Svedmyr et al. 1977; Ogilvie 1978; Hendeles and Weinberger 1980; Riegelman et al. 1980). Contributing to the interest in theophylline has been the discovery of several actions of this drug in the lung, e. g. inhibition of anaphylactic release of mediators (Andersson 1980), stimulation of mucociliary clearance (Matthus and Köhler 1980) and suppression of mediator-induced pulmonary oedema (Persson 1980). The wide clinical use has also highlighted the drawbacks of theophylline, particularly serious CNS toxicity at high plasma concentrations (Ogilvie 1978; Persson and Erjefält 1982), and great individual variation in plasma half life, because of clinically unpredictable rates of metabolism.

In the search for new antiasthmatic drugs, structure-activity studies suggested 3-propylxanthine (enprofylline, pINN) to be a promising candidate (Persson and Kjellin 1981). Due to their chemical resemblance, theophylline and enprofylline might be expected to have similar characteristics. However, fundamental differences between the two drugs were indicated by the preclinical studies. A highly significant cellular mechanism at therapeutic concentrations of theophylline, its blocking action at membrane receptors for adenosine (Rall 1980), is not shared by enprofylline (Persson et al. 1982). Probably as a consequence of this difference, enprofylline does not produce theophylline-like CNS-stimulation or diuretic effects in animals (Persson et al. 1982). Enprofylline thus carries the potential of producing less serious side effects than theophylline. Furthermore, the animal studies suggested that the antiasthmatic characteristics of enprofylline would be at least comparable to those of theophylline (Persson and Kjellin 1981; Persson et al. 1982). On the other hand, others have suggested that adenosine antagonism is of importance for the therapeutic effect of xanthines in asthma (Marquardt et al. 1978; Fredholm 1980).

The aim of the present study was to examine whether enprofylline produced bronchodilatation in

0031-6970/82/0022/0395/$01.60

Table 1. Details of patients in the study

Patients		Age [years]	Weight [kg]	Duration of Illness [years]	Medication	Preinvestigation reversibility [%]	$FEV_{1.0}$, starting value		
							Day 1	Day 2	Day 3
UF	♀	59	50	11	OT, OP, IB, IA	25	0.55	0.60	0.60
TS	♂	58	94	14	OT, IB, Thyroxine	15	1.25	1.35	1.40
AB	♂	57	80	16	OT, OB, OP, IB, IG	40	0.75	0.75	0.75
TN	♂	69	70	35	OT, OB, IB, IG	30	1.20	1.15	1.20
HB	♂	69	100	41	OT, IB, IG	25	0.75	0.95	0.85
AJ	♂	68	85	11	OT, OP, IB, IG	40	1.05	1.10	1.00
ES	♂	64	74	11	OT, OB, IB, IG	27	1.25	1.10	1.20
KJ	♂	34	79	8	OB, IB	16	2.40	2.25	2.35

OT = Oral theophylline
OB = Oral beta stimulant
OP = Oral prednisolone
IB = Inhalation of beta-stimulant
IG = Inhalation of glucocorticoid
IA = Inhalation of anticholinergic drug

patients with reversible chronic obstructive lung disease, and if so, to compare this effect with that of theophylline in therapeutically recommended dosage. Additionally, some information was sought on the pharmacokinetics of enprofylline in patients. As supported by results in rats (Persson and Kjellin 1981) and human volunteers (Lunell et al. unpublished), renal excretion appears to be the sole mechanism of elimination of enprofylline.

Materials and Methods

There were 8 ambulant patients in the study, 7 men (ages 34–70 years; weight 64–100 kg) and 1 woman (59 years; 50 kg). The patients, who suffered from chronic, partly reversible, obstructive lung disease, were receiving maintenance treatment with bronchodilators and, in two cases, a low dose (5 or 10 mg) of prednisolone (Table 1).

Inclusion Criteria

To be accepted for the trial, the patient had to have reversible bronchial obstruction. Reversibility was defined as 15–50% increase in FEV_1 measured 5 min after the inhalation of 5 doses of Bricanyl® aerosol, each dose containing terbutaline sulphate 0.25 mg.

Exclusion Criteria

Fertile women, and patients suffering from severe heart, liver or kidney disease, or who had a history of migraine or seizures, were not accepted for the trial. Patients with ongoing infection were also excluded.

Study Design

The study was designed as a double blind, randomised cross over trial. Each patient was studied on three consecutive days. Day 2 was single blind placebo-day and Days 1 and 3 were randomised between theophylline and enprofylline.

Enprofylline (Batch No. F2) dissolved in a water drink, was administered orally together with placebo tablets. The dose of enprofylline was 2.0 mg/kg (10.3 μmol/kg) at time 0, and 4.0 mg/kg (20.6 mg/kg) at 120 min, the total dose being 6.0 mg/kg (30.9 μmol/kg). The choice of these doses of enprofylline was based on results obtained in a pilot study in two asthmatic patients.

The bitter taste of theophylline solution precluded its use in a double blind study. Therefore, theophylline was given as Theolair® tablets (125 mg); Batch No. FR 225, Riker Benelux) together with a water drink. The dose of theophylline was about 4 mg/kg (22 μmol/kg) at time 0 and about 6 mg/kg (33 μmol/kg) at 120 min, the total dose approximating 10 mg/kg (55 μmol/kg).

On Day 2 placebo tablets and a water drink were administered. All oral medication except glucocorticoids was withheld 56 h before the start of the study. Inhalation treatment with sympathomimetics, anticholinergics and glucocorticoids was withheld form 22.00 h on the evening before Day 1: it was allowed again between 17.00 and 22.00 h on the three trial days.

FEV_1 and vital capacity were measured 60 min and immediately before intake of drug (time 0 = basal value), and then every 30 min for 360 min. ECG, heart rate, arterial blood pressure and hand tremor were recorded at the same time as the spirometry, and the patient was asked about side effects according to a pre-determined protocol.

Each trial day was closed by performing the same measurements 5 min after the inhalation of 5 doses of Bricanyl® aerosol, each dose containing terbutaline sulphate 0.25 mg.

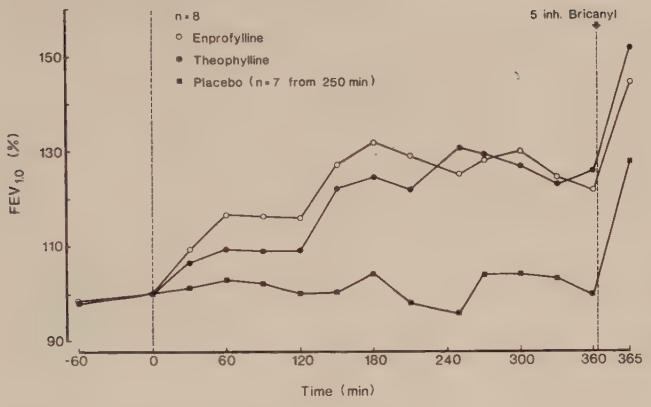

Fig. 1. Changes in mean $FEV_{1.0}$ in 8 patients after enprofylline, theophylline and placebo; at the end of each day the patient inhaled 5 doses of Bricanyl® aerosol

Table 2. Effects on FEV_1 in per cent relative to the starting value (at time 0), mean individual changes [%] relative to the starting value (at time 0) and relative to the value obtained during placebo treatment

Time [min]	Enprofylline/ placebo difference	Theophylline/ placebo difference	Enprofylline/ starting value difference	Theophylline/ starting value difference
0				
30	8.0	5.3	9.3[a]	6.6
60	13.8[b]	6.5[a]	16.7[c]	9.4
90	14.1[a]	6.8	16.2[c]	8.9
120	16.0[a]	9.1[a]	16.0[a]	9.1
150	27.0[b]	21.8[b]	27.3[c]	22.1[a]
180	27.6[b]	20.4[a]	32.0[c]	24.7[a]
210	31.1[c]	24.1[b]	29.1[c]	22.1[c]
250	29.5[b]	35.2[a]	25.4[c]	31.0[b]
270	23.2[c]	22.9[b]	28.4[c]	29.6[b]
300	25.1[c]	19.1[a]	30.2[c]	27.2[b]
330	21.5[b]	19.5[a]	24.7[c]	23.4[b]
360	24.5[b]	23.3	22.1[b]	26.1[a]
Inh. Bricanyl	17.3[b]	20.1	45.0	52.3[b]

$N = 8$ except for the final five measurements during the placebo day when $n = 7$ (see text)
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$

Standard Laboratory Tests

Blood and liver tests, serum creatinine, and urine examinations were made on the first trial day before drug intake and were repeated after 7–15 days.

Calculations

P-values of the difference from 0 were calculated using a two-sided *t*-test. Any effect on FEV_1 is reported in the text as the maximum mean effect. Plasma levels are given as the mean of the individual maximum values. Dispersion is shown as the standard deviation.

Analysis of Drugs in Plasma and Urine

A detailed description of the analytical procedure used for unchanged enprofylline in plasma and urine is given elsewhere (Edholm and Heintz unpublished; cf Edholm 1980).

High pressure liquid chromatography was used, with chemically bonded reversed-phase material of C_{18}-type. High pressure liquid chromatography was also used to analyse theophylline in plasma (cf. Svedmyr et al. 1977).

Results

Spirometry

Oral intake of enprofylline 2 mg/kg produced a mean maximum increase in FEV_1 of 17% ($p < 0.001$) at 60 min. An additional dose of enprofylline 4 mg/kg given 120 min after the 2 mg/kg dose, produced a further improvement in FEV_1, which had increased 50 min later to a mean maximum of 32% ($p < 0.001$) compared to the basal value (Fig. 1). At the end of the experiment 5 doses of Bricanyl® aerosol were administered, resulting in a maximum FEV_1 45% above the basal value (Fig. 1).

Theophylline 3.9 ± 0.7 mg/kg produced a maximum increase in FEV_1 of 9% (not statistically significant) compared to the basal value. An additional dose of theophylline was administered 120 min after the

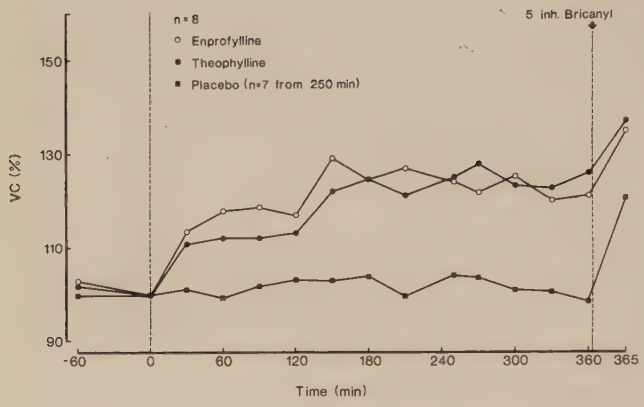

Fig. 2. Changes in mean vital capacity after enprofylline, theophylline and placebo

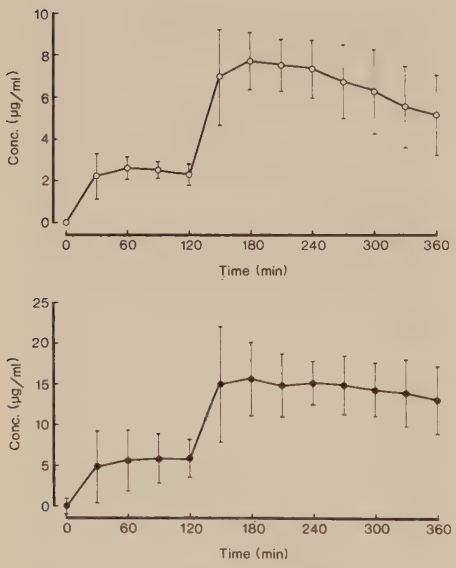

Fig. 3. Mean plasma concentrations of enprofylline ○—○ and theophylline ●—● after intake of 2 + 4 mg/kg and 4 + 6 mg/kg, respectively

first one, giving a total theophylline intake or 9.2 ± 0.9 mg/kg. The mean maximum improvement after theophylline was 31% ($p < 0.01$; Fig. 1). Theophylline plus five doses of Bricanyl® aerosol produced a 52% increase in FEV_1 over the basal value.

The effects on FEV_1 of enprofylline and theophylline compared to placebo treatment and starting values are shown in Table 2. Enproylline significantly improved the FEV_1 compared to placebo at all times, except at 30 min after drug intake (Table 2). After intake of theophylline a significant improvement in FEV_1 compared to placebo was recorded at all times except at 30, 90 and 360 min (Table 2).

The changes in vital capacity corresponding to those in FEV_1 occurring after enprofylline and theophylline are illustrated in Fig. 2.

No improvement in spirometric values was recorded after intake of placebo. One patient, who became acutely ill and had to be treated with bronchodilator aerosol (Bricanyl®) at 260 min, has been excluded from the study (Table 2).

Significant changes in FEV_1 and vital capacity occurred during the placebo day only after the inhalation of terbutaline. Five doses of Bricanyl® aerosol then produced an improvement of the same order as that achieved by enprofylline or theophylline alone (Figs. 1 and 2).

Plasma Concentrations of Drugs

The mean plasma concentrations of enprofylline and theophylline at the various sampling times are shown in Fig. 3. Enprofylline 2 mg/kg produced a maximum concentration of 3.0 ± 0.6 µg/ml (15.4 ± 3.1 µmol/l); the additional dose of 4 mg/kg produced a maximum plasma concentration of 8.5 ± 1.4 µg/ml (43.8 ± 7.2 µmol/l). The two dose levels of theophylline produced maximum plasma concentrations of 7.2 ± 3.2 µg/ml (40 ± 18 µmol/l) and 18.5 ± 4.7 µg/ml (103 ± 26 µmol/l), respectively.

Table 3. Renal elimination of enprofylline

Patient	Administered dose [mmol]	Total amount unchanged drug found in urine sampled for 6 h [mmol]	[%] of given dose	Max. conc. in urine [mmol × l^{-1}]
UF	1.55	0.95	61.8	3.08
TS	2.91	1.49	51.2	1.67
AB	2.47	1.27	51.2	5.25
TN	2.16	1.05	48.3	1.31
HB	3.09	1.07	34.8	3.38
AJ	2.63	0.93	35.5	2.23
ES	1.98	1.47	74.6	1.98
KJ	2.44	1.38	56.3	1.73
mean ± SEM	2.40 ± 0.2	mean ± SEM 1.20 ± 0.1	mean ± SEM 51.7 ± 4.6	mean ± SEM 2.58 ± 0.5

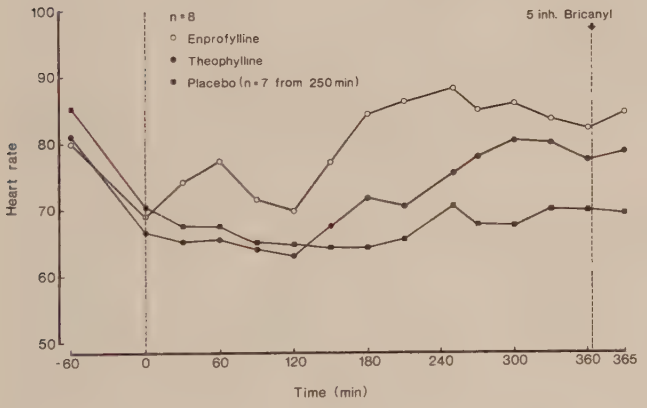

Fig. 4. Changes in mean heart rate after enprofylline, theophylline and placebo

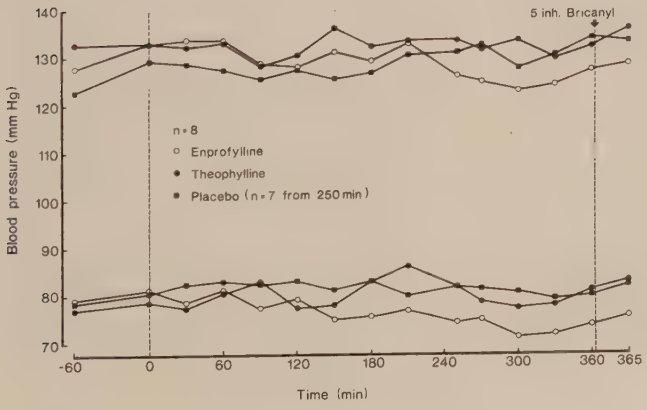

Fig. 5. Changes in mean blood pressure after enprofylline, theophylline and placebo

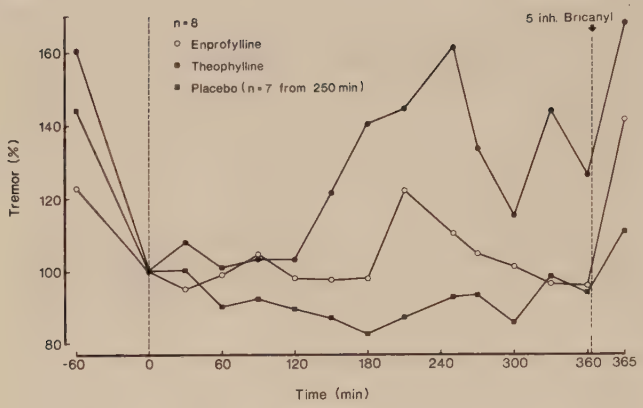

Fig. 6. Changes in mean tremor of hand after enprofylline, theophylline and placebo

Recovery of unchanged enprofylline in urine collected for 6 h after drug intake ranged from 35–75% (Table 3).

Cardiovascular effects

The effects of enprofylline and theophylline on heart rate and systolic and diastolic blood pressures are illustrated in Figs. 4 and 5. Theophylline produced significantly less effect on heart rate than enprofylline. The cardiovascular effects of enprofylline were not noticed by the patients. ECG-recordings showed nothing abnormal.

Tremor of Hands

There was a tendency to a greater percentage increase in tremor of the hands after theophylline compared to enprofylline. The effects of the drugs were small compared to individual variations (Fig. 6).

Side Effects

60 min after enprofylline 2 mg/kg a brief flush was recorded in one patient. The same reaction was also observed in this patient after taking the high dose of enprofylline. Enprofylline 2 mg/kg was otherwise free from side-effects. After the high dose of enprofylline, 5 patients reported side-effects; 3 had a headache that lasted 5–6 h, and 2 others experienced brief (15–20 min) nausea and epigastric discomfort. Three patients had no side-effects. Intake of the low dose of theophylline was associated in one case with palpitations, but otherwise no side-effects were found with this drug.

Standard Laboratory Tests

Laboratory tests in the follow-up period of the trial did not show any changes attributable to the drugs.

Discussion and Conclusions

The reversibility of the bronchial obstruction in the present patients was tested by inhalation of the β_2-receptor agonist terbutaline before the study and also on each day of investigation at the end of the experiment. The intra-individual variation in starting values on the different days of testing was acceptable. This demonstrated that there was an adequate margin for improvement by the drugs in FEV_1 and vital capacity.

The present study showed that enprofylline had a bronchodilating effect in man, comparable to that of theophylline, as judged by improvements in FEV_1 and vital capacity. At a dose of enprofylline of 2 mg/kg, producing a mean plasma concentration of 3.0. μg/ml, bronchodilatation was statistically significant (17%), and further improvement (32%) was obtained after addition of 4 mg/kg, producing a mean maximum plasma concentration of 8.5 μg/ml. Theophylline in doses producing plasma concentrations of 7.2 and 18.5 μg/ml, caused improvements of 9 and 32%, respectively. Comparison of these data supports the view that the approximate potency ratio of between four and five to one between enprofylline and theophylline found in in vitro and in vivo animals for the bronchodilator effect (Persson and Kjellin 1981, is also valid in man. No difference in maximum effect was observed, probably because the highest doses given produced bronchodilator effects near the maximum obtainable with xanthines.

Preclinical results have indicated a fundamental difference between enprofylline and theophylline. Enprofylline, due to its lack of alkyl substitution in the l-position, seems to be devoid of adenosine-receptor antagonism (Persson et al. 1982), a mechanism implicated in many pharmacological actions of theophylline (Bäer and Drummond 1979). However, the present finding that, in agreement with animal data (Persson and Kjellin 1981), enprofylline is a potent bronchodilator in man, does not favour the suggested role of adenosine antagonism as a mechanism in the antiasthmatic effects of xanthines (cf. Fredholm 1980).

The maximum increase in heart rate was higher after both the low and high doses of enprofylline than after those of theophylline. This may reflect differences in the cardiovascular effects of the drugs, but it might also be attributable to the fact that doses producing equibronchodilator effects were not given. In the low dosage, enprofylline had twice the bronchodilator effect of theophylline. It cannot be excluded that the high dose of enprofylline was higher than necessary to produce a maximum bronchodilator effect, thus giving a less favourable ratio between bronchodilatation and increase in heart rate than for theophylline. The higher frequency of side effects with enprofylline may also be attributable to the high dose of this drug.

Animal data suggest that the ratio between bronchorelaxant and cardioaccelerating effects, studied in the probable absence of any influence of adenosine, is either slightly less for enprofylline or approximately the same as that found for theophylline (Persson 1982; Persson and Erjefält, unpublished observations). Adenosine antagonism by theophylline may, per se, induce cardiovascular effects because, and adenosine itself is a potent vasodilator (Bäer and Drummond 1979). Some differences in cardiovascular effects can therefore be expected, due to the different abilities of the compounds to antagonise adenosine. These possibilities will have to be studied in specially designed investigations.

Tremor of the hand tended to be higher after theophylline than after enprofylline. However, as these recordings showed large interindividual variation, no valid conclusion can be drawn about possible differences in effect. Tremor and jitteriness may be produced by theophylline as a consequence of its CNS-stimulant effects (Andersson and Persson 1980). The mechanism behind both slight and massive CNS-stimulation by theophylline is considered to be antagonism of the depressant effects of adenosine on CNS neurons (Rall 1980). It would be of particular interest therefore, further to examine and compare the tremor-inducing effects of theophylline and enprofylline.

Previous pharmacokinetic information on enprofylline in man (Lunell et al. unpublished) has shown that the drug is rapidly and almost completely absorbed after oral intake, has a plasma half-life of approximately 2 h, and is excreted unchanged in the urine. These findings were supported by the results of the present study. Maximum or near maximum plasma levels were obtained within 60 min after intake, and within 6 h 36–75% of the given dose was recovered in urine as unchanged drug.

In conclusion, enprofylline which is a xanthine derivative lacking blocking actions at a number of adenosine receptors, is a potent bronchodilator. However, the differences from theophylline at the cellular level indicate, as shown in animal studies, that enprofylline is devoid of certain characteristics of theophylline, particularly its CNS-stimulant effects (Persson 1982). These aspects, together with the fact that the drug may not be metabolised but rather is quantitatively eliminated by the renal route, makes enprofylline a new and promising alternative in the treatment of obstructive lung disease.

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Received: May 25, 1981
accepted: September 25, 1981

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VI

A Novel Bronchodilator Xanthine Apparently without Adenosine Receptor Antagonism and Tremorogenic Effect

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Abstract. Enprofylline is a potent bronchodilating xanthine that has been shown to have no or negligible ability to antagonize adenosine in a variety of cell types. In eight patients with chronic obstructive lung disease a single dose of enprofylline (3.5 mg/kg) was given orally. A double blind, randomized, crossover comparison was made with oral theophylline (9.5 mg/kg) that is a potent and general adenosine receptor antagonist. Forced expiratory volume (FEV_1), vital capacity, tremor of hands, heart rate, and blood pressure were followed for 6 hours. Plasma concentrations of both drugs were monitored. At doses producing significant bronchodilation, theophylline caused a significant increase in tremor of hands whereas enprofylline did not. There were no significant changes in heart rate or blood pressure after either drug.

It is suggested that the lack of tremorogenic effect of enprofylline is related to its lack of CNS-stimulant behavioural effects and hence may reflect its low ability to antagonize nervous depressant actions of adenosine.

Key words: Adenosine antagonism - asthma therapy - bronchodilation - CNS-stimulant effect - enprofylline - plasma concentration - theophylline - tremorogenic effect - xanthines.

Theophylline is a methylxanthine which has been shown to interfere with a number of mechanisms at the cellular level; it mobilizes intracellular calcium, inhibits phosphodiesterase enzymes, and antagonizes adenosine receptor stimulation (11). The blockade of adenosine receptors is pronounced already at therapeutic concentrations of theophylline normally obtained in patients (2,11), and many pharmacological effects are presently being attributed to this mechanism (2,5,8,11). Despite suggestions to the contrary (cf 2), the bronchodilator effects of xanthines may not reflect adenosine antagonism (5,6,7). Structure-activity studies indicate that adenosine antagonism can be differentiated from the bronchodilator activity of xanthine derivatives (5,6,7,9). A novel xanthine derivative, enprofylline (10) has been shown to be a potent bronchodilator, yet it seems to be practically devoid of adenosine antagonism in smooth muscle, cardiac muscle and nervous tissue (7,9). In animal experiments enprofylline has been shown to be without the theophylline-like diuretic and CNS-stimulating behavioural effects (5,6,7,8).

Studies in animals and experiments with isolated human bronchi have indicated that enprofylline is about 5 times as potent as theophylline as a bronchodilator (7,9,10). Preliminary evaluation in patients suggested that enprofylline produced significant bronchodilation already at plasma concentrations of 3 µg/ml (3) and that enprofylline may not share with theophylline a tremor-inducing ability. This second study of enprofylline in patients was performed to examine bronchodilating and tremor-inducing effects of enprofylline. Comparisons were made with theophylline.

Experimental design

The study was performed as a double blind, randomized, cross-over trial. On two consequtive days oral doses of enprofylline and theophylline were given. Effects on ventilatory capacity,

circulation and skeletal muscle tremor and plasma levels of the drug were recorded. The study was approved by the Ethical Committee of the University of Göteborg.

Patients

In the study participated 8 adult male outpatients with intrinsic asthma aged 34-69 years and with a duration of illness of 4-41 years. All had earlier taken part in similar studies and had a stable bronchospasm when off treatment. Clinical data including drug treatment are summarized in Table 1. The patients had 20-50% reversibility in FEV_1 after 5 terbutaline inhalations (1.25 mg terbutaline sulphate, Bricanyl^R). All patients entered the study although two of them had basal respiratory values that differed more than 15% between the two test days (Table 1). All except one had normal heart and liver function. This patient (No. 5) was on maintenance therapy with digoxin and quinidine because of previous attacks of atrial fibrillation.

Theophylline was withdrawn for 56 hours, β-adrenoceptor stimulants, beclometasone dipropionate inhalations and anti-cholinergic drugs for at least 11 hours. Patients on oral steroids were allowed to continue this medication in the same dose as before the study.

Experimental details

The patients had a light breakfast (without coffee, tea or cola drinks) not later than 2 hours prior to the drug administration. After arrival at the laboratory, the patients were placed in a comfortable chair in a semirecumbent position. After 60 min rest basal values of the following variables were measured:

- Forced expiratory volume in one second (FEV_1) and forced vital capacity, both with a Bernstein spirometer
- Skeletal muscle tremor recorded according to Thiringer and Svedmyr (14) with a single plane accelerometer, model SPA 1 (Grass). The device with its insulation was fixed

TABLE 1.

Data on the patients. Reversibility prior to the trial, and basal values of tremor* and FEV_1 (L) after 60 minutes rest on the two trial days

Patient	FEV_1 reversibility after 5 inhalations of terbutaline	Theophylline day		Enprofylline day	
		Tremor	FEV_1	Tremor	FEV_1
No 1	1.00 → 1.45	103	1.00	76	1.25
" 2	1.10 → 1.35	219	1.05	273	1.20
" 3	1.15 → 1.50	45	1.15	58	1.20
" 4	0.80 → 0.95	158	1.00	174	1.10
" 5	1.50 → 1.85	197	1.05	195	1.20
" 6	1.35 → 1.55	162	1.35	160	1.45
" 7	1.50 → 1.95	60	1.20	75	1.45
" 8	2.25 → 2.75	57	2.30	67	2.40

*Absolute amount of tremor obtained by measuring the integrated amplitudes in mm (cf ref 14)

to the right hand third finger. Tremor oscillations were recorded (Grass polygraph) as such and via an integrator (Grass) which summed up the deflections for each 10 seconds period. The values were expressed as absolute amount or as tremor ratios where each individual patient's basal tremor was set to 1.00.

- Heart rate employing a continuous ECG.
- Blood pressure with the cuff method and a mercury manometer.

The two treatments were given randomly at day A and day B as follows:

A) Rapidly dissolving tablets of theophylline (60.1 mg, batch DF C7, Draco) in a dose of 9.5 ± 0.2 mg/kg (53 ± 1 µmol/mg) mean ± SD.

B) Tablets of enprofylline (57.7 mg, batch DF C5, Draco) of the same quality and appearance as above, in a dose of 3.5 ± 0.2 mg/kg (18.0 ± 0.8 µmol/kg).

The dose regimens were selected because pilot studies with the tablets suggested that plasma levels between 10-20 µg/ml for theophylline and 2-4 µg/ml for enprofylline would be produced. Bronchodilator equivalence at these levels is supported by animal data and results obtained in isolated human bronchi (7,9,10).

All the above variables were recorded and plasma samples were drawn every 30 min for 360 min after drug intake. The patients were regularly questioned about side effects according to a protocol. At 180 min after intake of the drugs the patients were given a meal consisting of two sandwiches and a glass of milk.

Each trial was closed by performing the measurements after 5 doses of Bricanyl^{R} aerosol (each dose containing 0.25 mg terbutaline sulphate).

Foot note: Tablets. Rapidly dissolving tablets of enprofylline and theophylline were produced at Draco (N.O. Lindberg).

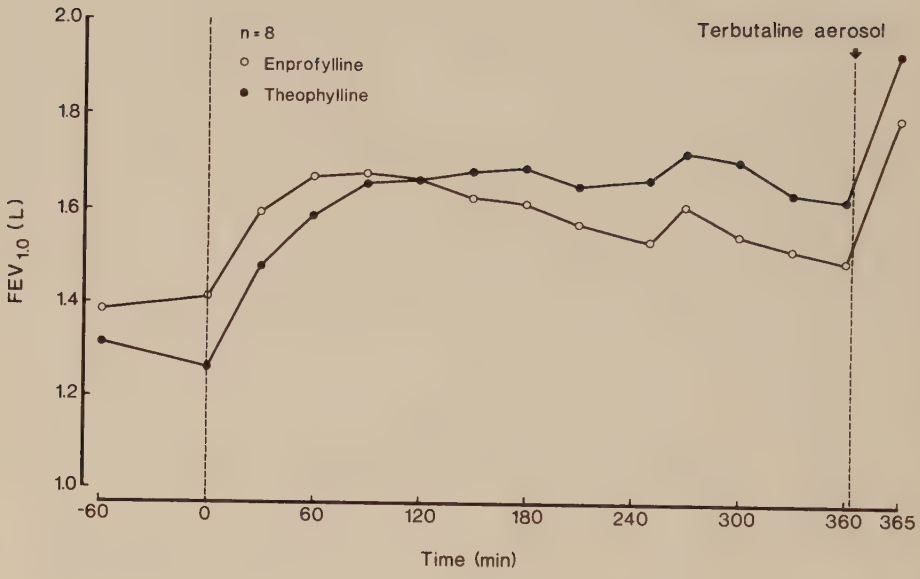

Fig. 1. Effects on FEV_1 after intake (at time 0) of enprofylline and theophylline.

Standard laboratory tests. Blood and liver tests, serum, creatinine and urine examination were performed before drug intake and were repeated after 7-14 days.

Analysis of drugs in plasma and urine. A detailed description of the analytical procedure used for unchanged enprofylline in plasma is given elsewhere. The analyses of enprofylline and theophylline were performed at AB Draco (L.-E. Edholm) and at the Sahlgrenska Hospital (T. Mellstrand) respectively, using high pressure liquid chromatography (HPLC) for both drugs.

Statistics. P-values were calculated using a two-sided t-test of the difference from O. Dispersion is given as standard error of the mean.

Results

Spirometry

Both enprofylline and theophylline had a rapid onset of action, producing near maximum spirometric effects within 90 min (Figure 1). Despite randomization all starting values were lowest on the theophylline days.

Enprofylline in a dose of 3.5 ± 0.2 mg/kg increased FEV_1 from a starting value of 1.41 ± 0.42 L to a maximum of 1.67 ± 0.51 L ($p \leq 0.01$) 90 min after intake. When Bricanyl^R aerosol was given 360 min after intake of enprofylline, FEV_1 increased to a maximum of 1.78 ± 0.51 L. Theophylline in a dose of 9.5 ± 0.2 mg/kg increased FEV_1 from a starting value of 1.26 ± 0.44 L to a maximum of 1.74 ± 0.56 ($p < 0.01$) 270 min after intake. With Bricanyl^R aerosol, FEV_1 increased to 1.92 ± 0.59 L.

The changes in vital capacity followed the same pattern as those in FEV_1. All patients except one had recently participated in a study showing no improvement during 6 hours following intake of placebo (3).

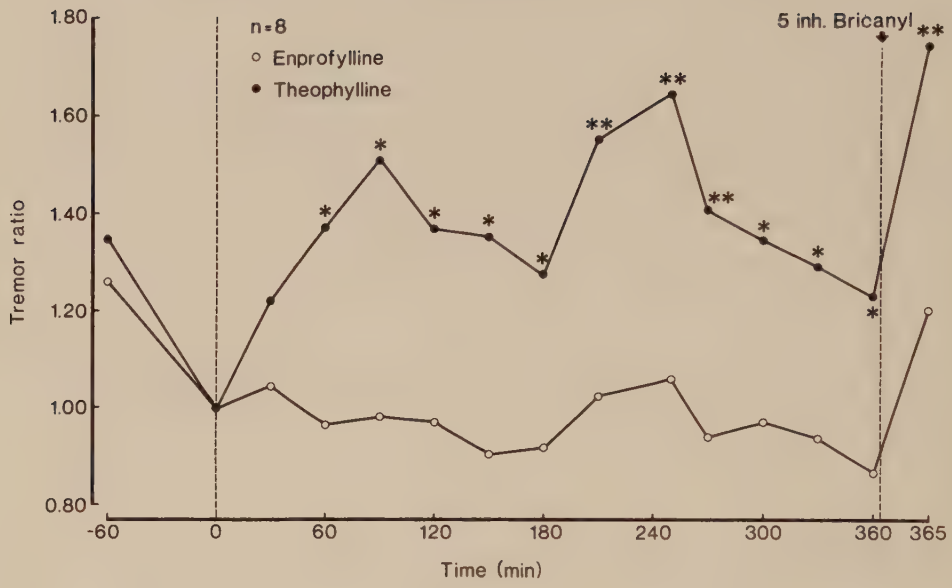

Fig. 2. Effects on mean tremor ratio by theophylline and enprofylline. Each patient's basal tremor was set to 1.00. The two maximum ratios were 1.51 ± 0.14 (mean ± SEM) at 90 minutes and 1.64 ± 0.20 at 250 minutes after intake of theophylline. The corresponding values recorded after enprofylline were 0.98 ± 0.08 and 1.06 ± 0.10, respectively. Asterisks denote significant differences between theophylline and enprofylline. * = $p < 0.05$ ** = $p < 0.01$

Tremor

After intake of enprofylline the tremor did not change from the basal value. Theophylline produced a significant increase in tremor of hands compared both to starting values and to values recorded after intake of enprofylline (Figure 2). When the patients were given the meal at 180 min after intake of the drug, there was an additional increase in tremor. The 5 inhalations of Bricanyl^{R} aerosol at the end of the experiment produced an increment in tremor above that induced by theophylline.

Cardiovascular effects

Enprofylline and theophylline were without significant effects on heart rate and systolic and diastolic blood pressure. Patient No. 3, well known to the staff of the department, often had frequent extrasystoles when participating in bronchodilator trials. He had frequent extrasystoles, recorded on ECG 90-120 min after intake of theophylline but not after enprofylline.

Plasma concentrations of drugs

Mean plasma concentrations of unchanged enprofylline and theophylline are illustrated in Figure 3. Enprofylline produced a maximum mean concentration of 3.0 ± 0.4 µg/ml 120 min after drug intake. Theophylline produced a maximum mean concentration of 16.0 ± 0.8 µg/ml 150 min after drug intake.

Subjective side effects

Patient No. 6 had a transient, slight nausea 90-120 min after intake of enprofylline. The only patient (No. 8) who was not on maintenance theophylline therapy, experienced nausea on the first trial day, 60-180 min after intake of enprofylline. The nausea was accompanied by occasional dizziness 60 min after intake.

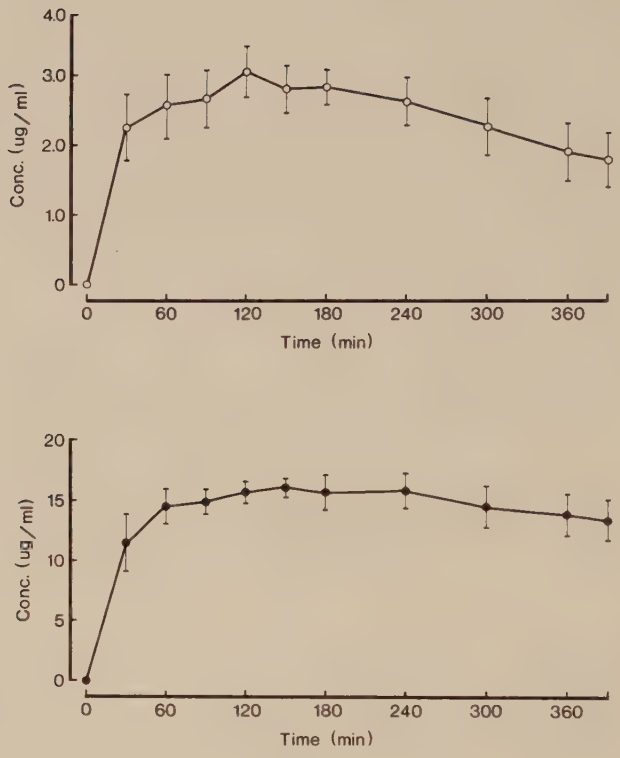

Fig.3 Plasma concentrations of enprofylline (upper curve) and theophylline (lower curve). Vertical lines show ± SEM. S. I. conversion: µmole/L = µg/ml. 5.1.

Patient No. 4 had a short-lasting epigastric pain 30-60 min after intake of both enprofylline and theophylline and nausea and vomiting 6-8 hrs after intake of theophylline. He had a history of recurrent duodenal ulcers and frequent use of antacids. Patient No. 5 had a short-lasting (30 min) headache 60 min after intake of enprofylline. Patient No. 6 experienced tremor 90 and 270 min after intake of theophylline and also after the 5 inhalations of terbutaline.

Standard laboratory tests

Laboratory tests in the follow-up of the trial were without changes that could be related to the drugs.

Discussion

Enprofylline, and theophylline at optimum recommended plasma concentrations, produced significant bronchodilation. A direct comparison between the two drugs is hampered by different mean basal values in the spirometry on the two days of experiment. Also, a number of different plasma levels are needed to assess the correct potency ratio between enprofylline and theophylline in asthmatics. This question will be addressed under controlled conditions of i.v. infusion. In the present study the mean plasma levels of the two xanthines differed by a factor of about five. The possibility that these concentrations exhibit approximate bronchodilator equivalence is supported by animal data and results from isolated human bronchi showing that enprofylline is about five times more potent than theophylline as a broncodilator (7,9,10).

Tremor after theophylline is frequently reported as a subjective side effect (1,11). There was one report, however, that indicated an objectively verified tremor-increase after a large intravenous dose of theophylline (12).

The present study showed that oral theophylline produced significant tremor of hands in patients. The onset and the

intensity of the tremor were correlated to the plasma level of the drug. Intake of a meal starting at 180 min ,(Figure 2) slightly increased the tremor. An interesting finding was also that tremor induced by inhalation of a β-receptor stimulant added to that produced by theophylline.

Available information suggests that tremor by methylxanthines, such as theophylline, is a sign of their CNS--stimulant behavioural effects (1,4,8,11) and that these are due to adenosine receptor antagonism (8,9,11). Experimental data do not support the possibility that xanthines would produce tremor via phosphodiesterase-inhibition (11, 13).

Enprofylline, in the given dose, produced marked bronchodilation but was without tremorogenic effects. This finding tallies with observations in animals where enprofylline was found to be devoid of CNS-stimulant behavioural effects. Thus enprofylline did not produce an increase in locomotor activity (9) nor did it induce theophylline-like seizure activity in a number of animal species (8). Enprofylline also lacked the ability of theophylline to antagonize nervous depressant effects of adenosine (9). It is, therefore, suggested that the present findings of differences in tremorogenic actions between enprofylline and theophylline may reflect differential effects in the CNS, presumably at adenosine receptors.

A wide range of i.v. and oral doses of enprofylline has been given to healthy volunteers, who did not experience any CNS-stimulant behavioural effects such as restlessness, lightheadedness or irritability (unpublished work by Lunell et al.). Furthermore, high plasma levels of enprofylline (8.5 ± 1.4 µg/ml, mean ± SEM) in asthma patients were not associated with such CNS-symptoms (3). Headache and nausea may be seen after both enprofylline and theophylline, suggesting that the mechanism behind headache and nausea should differ from that behind the behavioural actions (cf. 8).

Theophylline and enprofylline given as tablets were rapidly and well absorbed. Enprofylline has a shorter plasma half-life than theophylline, but the plasma levels of both xanthines were well sustained during the course of the experiments. Under these conditions minor differences in the rate of drug distribution to the brain would not be of significance. In guinea-pigs receiving continuous infusions of enprofylline or theophylline, high brain levels of both xanthines were established within an hour (cf. 8)

There was no significant effect on heart rate by either xanthine. This is in accordance with the previous clinical studies with theophylline and with preclinical data on the heart rate effects of enprofylline and theophylline (7,10). The lack of cardiovascular effects might have been of importance for the tremor recording which can be influenced by chronotropic effects (14).

In conclusion, the present study showed that enprofylline and theophylline, in doses which produced bronchodilation, differed in their ability to produce tremor. The lack of tremorogenic effect of enprofylline may be attributed to its low ability to antagonize adenosine.

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VII

Intravenous Enprofylline in Asthma Patients

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Abstract. Enprofylline is a novel xanthine derivative with negligible adenosine antagonizing ability. It is eliminated almost exclusively by renal clearance with a half-life of about 2 hours. Enprofylline was given i.v. to eight patients with partially reversible chronic airway obstruction in a double blind cross-over trial with placebo. Three infusions of drug (1 mg/kg in 10 min) were given at hourly intervals. Spirometry ($FEV_{1.0}$ and VC) and drug analyses showed that enprofylline produced significant and concentration-dependent bronchodilation between plasma levels of 1 and 4 mg/L. The maximum effect obtained was similar to that produced by five doses of terbutaline aerosol. Heart rate was significantly increased by enprofylline. No change in tremor of hand and no subjective side effects were recorded. It is concluded that enprofylline given i.v. is well tolerated and produces marked and dose-dependent bronchodilation.

Key words: Enprofylline - intravenous administration - bronchodilation - cardiovascular effects - tremor - asthma patients.

INTRODUCTION

Since its entry into bronchopulmonary medicine (4), theophylline has been an important intravenous (i.v.) drug in the treatment of acute asthma. The accumulated experience with theophylline has made possible a more effective treatment of asthma, but also highlighted some clinical disadvantages with this xanthine derivative. About 90 % of a given dose of theophylline is eliminated by metabolism, the rate of which can vary greatly between and within individuals (5). Toxic theophylline levels may thus be produced due to unpredictable, long half lives. A xanthine bronchodilator less dependent on the metabolizing capacity of the body and which is eliminated by renal excretion, could be an interesting i.v. alternative. Enprofylline (fig. 1) seems to have these properties (6,10,13). Furthermore, enprofylline seems to lack a number of theophylline's extra-pulmonary effects, such as diuretic and CNS-stimulant behavioural actions (restlessness, seizures). This difference has been suggested to reflect the potent ability of theophylline and the poor ability of enprofylline to antagonize several actions of adenosine (8,9,10,12) (Fig. 1).

This study reports on the initial experiments with i.v. enprofylline in patients with asthma. In a placebo-controlled double-blind acute multiple-dose study bronchodilation (spirometry), changes in heart rate, arterial blood pressure and tremor were recorded at three different plasma levels of enprofylline.

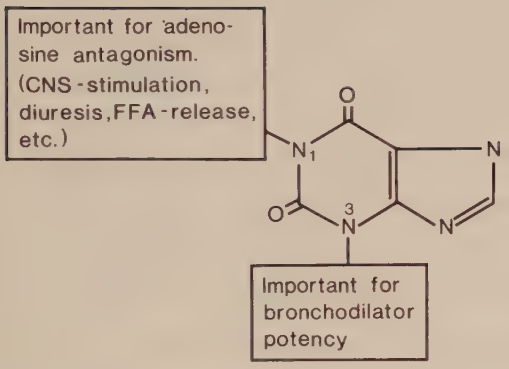

Fig. 1. Structure-activity relationships based on findings with enprofylline, theophylline and other xanthines (8). Alkyl in the 1-position as with theophylline is important for adenosine receptor antagonism. Many extrapulmonary effects of theophylline may be linked to this mechanism. By avoiding substitution in the 1-position and optimizing the alkyl-substitution in the 3-position potent and relatively lung selective xanthines can be produced. This is illustrated by the profile of action of enprofylline (12). Xanthines substituted in the 3-position only seem to depend on renal excretion solely rather than metabolism for their elemination (8).

METHODS

Patients

Eight adult outpatients, aged 45-70 years and with an asthma history of 5-40 years, participated in the study. All had taken part in similar studies previously and had a stable and reproducible bronchial obstruction when off treatment. They had 20-80 % reversibility in $FEV_{1.0}$ after five inhalations of 0.25 mg terbutaline sulphate (Bricanyl^{R}, Astra Pharmaceuticals). Basal $FEV_{1.0}$ values differed by 15 % or less between test days calculated as % of the highest value. All had normal heart, liver and kidney functions except one patient, who had a chronic atrial fibrillation. None were suffering from acute infection.

Before the study, theophylline and oral β-adrenoceptor stimulants were withdrawn for 56 h. Inhalations of bronchodilators and glucocorticoids were withdrawn for at least 11 h. One patient on oral corticosteroids was allowed to continue this medication in unchanged dosage. Coffee, tea or cola drinks were not consumed 24 h before the start of the study. The study protocol was approved by the ethical Committee of the University of Gothenburg, and all patients gave informed written consent.

Procedure

The study had a randomized double-blind cross-over design. The patients had a light breakfast, at least 2 h prior to drug administration. After arrival at the laboratory, they were placed in a comfortable chair in a semirecumbent position. The following variables were measured, in the order below, at 8.00 a.m. and again after a 60 min rest:

- skeletal muscle tremor with an accelerometer method according to Thiringer and Svedmyr (15),
- heart rate from a continous ECG,
- systolic and diastolic blood pressure (mercury manometer),
- the patients were asked about side effects according to a protocol including tremor, palpitations, nausea and headache,
- forced expiratory volume in one second ($FEV_{1.0}$) and forced vital capacity (VC) with a Bernstein spirometer.

Three 10-min infusions of 1 mg/kg bodyweight of enprofylline (from a 10 mg/ml solution) or a matched placebo (isotonic saline solution) were given at 60-min intervals. All the above variables were recorded and plasma samples were drawn just before, at the end of and in between the infusions. After the last infusion, plasma samples were drawn at intervals up to 300 min after the first dose. At this time 5 puffs of terbutaline aerosol total of 1.25 mg terbutaline sulphate, were given. A light meal consisting of two sandwiches and a glass of milk was served after 180 minutes.

Drug analyses

Blood samples for determination of enprofylline were collected in heparinised tubes (Venoject^{R}) and centrifuged within 10 min. The samples of plasma were frozed within one hour and stored at -20^{o} C until analysed.

Enprofylline in plasma was determined by liquid column chromatography (HPLC), employing columns packed with reversed-phase material of the C_{18}-type. A mobile phase consisting of methanol/water 30/70 (v/v) containing one gram of phosphoric acid per litre was used. A detailed description of the analytical procedures is in preparation (Edholm & Heintz).
The method is specific for enprofylline, and the interassay variation expressed as the coefficient of variation is less than 3.5 % at 0.4 and also at 3.0 mg/L.

Statistical methods

All quantitative data are presented in the text as mean $\pm$ SEM. The differences between placebo and treatment values were evaluated by Student's paired t-test. Results corresponding to probabilities less than 0.05 were considered statistically significant.

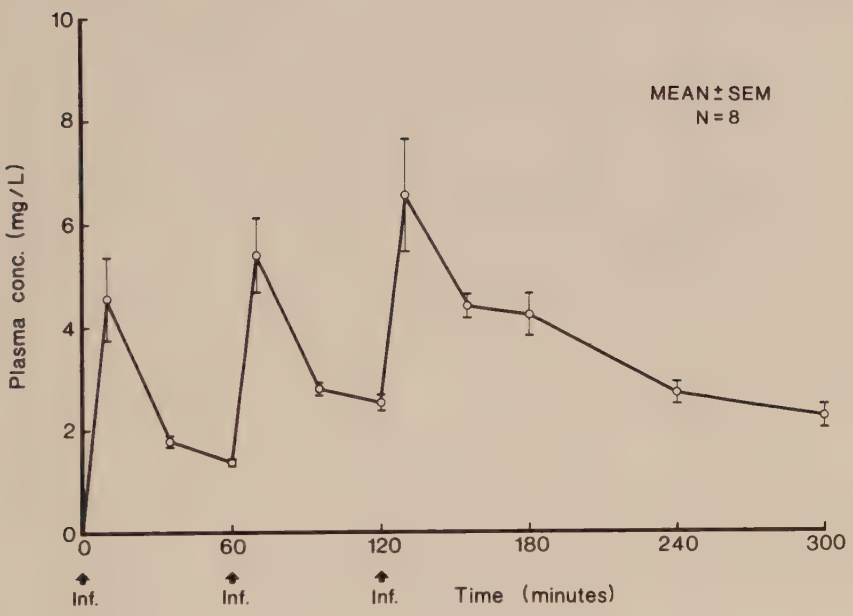

Fig. 2. Plasma concentrations after repeated 10-min infusions of 1 mg/kg of enprofylline in asthmatic patients.
S. I. conversion: umole/L = mg/L · 5.1.

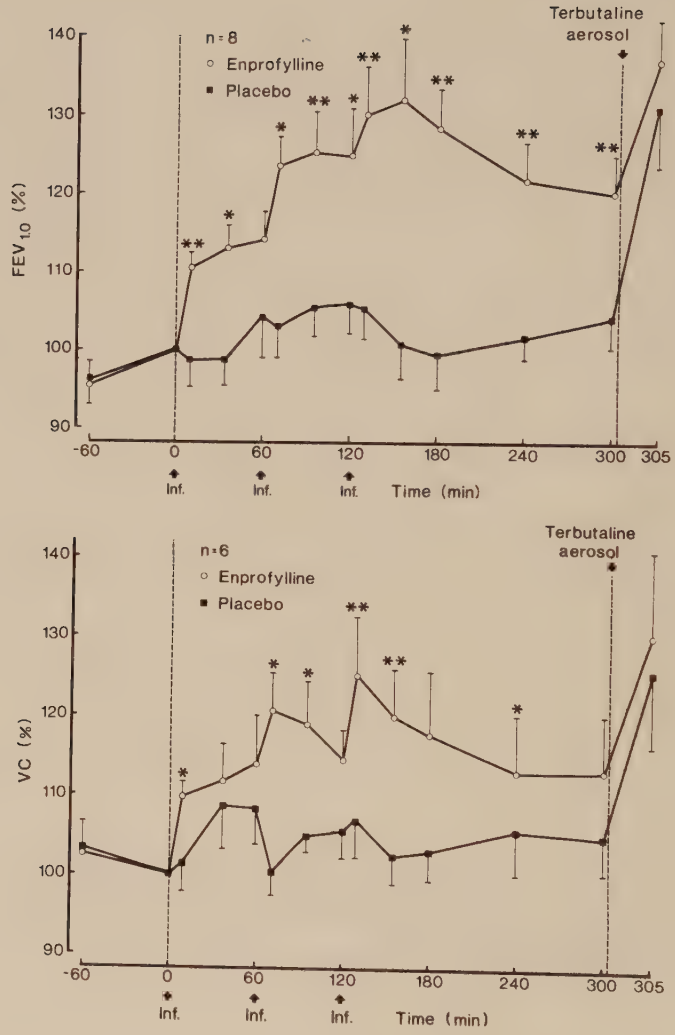

Fig. 3. FEV_1 and VC (% of basal values) after repeated 10 - min infusions of 1 mg/kg of enprofylline and placebo in asthmatic patients. Mean ± SEM are given.

* = $p<0.05$ ** = $p<0.01$

RESULTS

Plasma concentrations. The peak plasma concentrations of enprofylline amounted to 4.56 ± 0.8 mg/L (23.4 ± 4.1 µmol/L) after the first infusion, 5.41 ± 0.72 mg/L (27.8 ± 3.7 µmol/L) after the second infusion and 6.57 ± 1.09 mg/L (33.8 ± 5.6 µmol/L) after the third infusion (Fig. 2). Fifty min after the start of each infusion, when the distribution phase, according to the shape of the plasma concentration curve, appeared to be largely terminated, the plasma concentrations were 1.37 ± 0.06 mg/L, 2.54 ± 0.16 and 4.25 ± 0.41 mg/L (Fig. 2).

Ventilatory capacity The basal $FEV_{1.0}$ values were 1.28 ± 0.11 L on the placebo days and 1.36 ± 0.14 L on the enprofylline days. During the placebo days, the mean $FEV_{1.0}$ values did not differ significantly from the basal values at any time. Compared to the starting value $FEV_{1.0}$ was maximally increased by 31.8 ± 7.7 % after the third infusion of enprofylline. This effect was similar to the increase produced by inhalation of 1.25 mg terbutaline sulphate on the placebo days (Fig. 3).

The percent increases in $FEV_{1.0}$ after enprofylline infusions were apparently concentration-dependent and significantly larger than those after placebo (Figs 3,4). An additional effect was observed after inhalation of terbutaline on the enprofylline days. The percent increase in $FEV_{1.0}$ after terbutaline given at 300 min was 14.1 ± 2.2; $p<0.001$ (see Fig. 3). The effects on VC followed the same pattern as those on $FEV_{1.0}$ (Fig. 3).

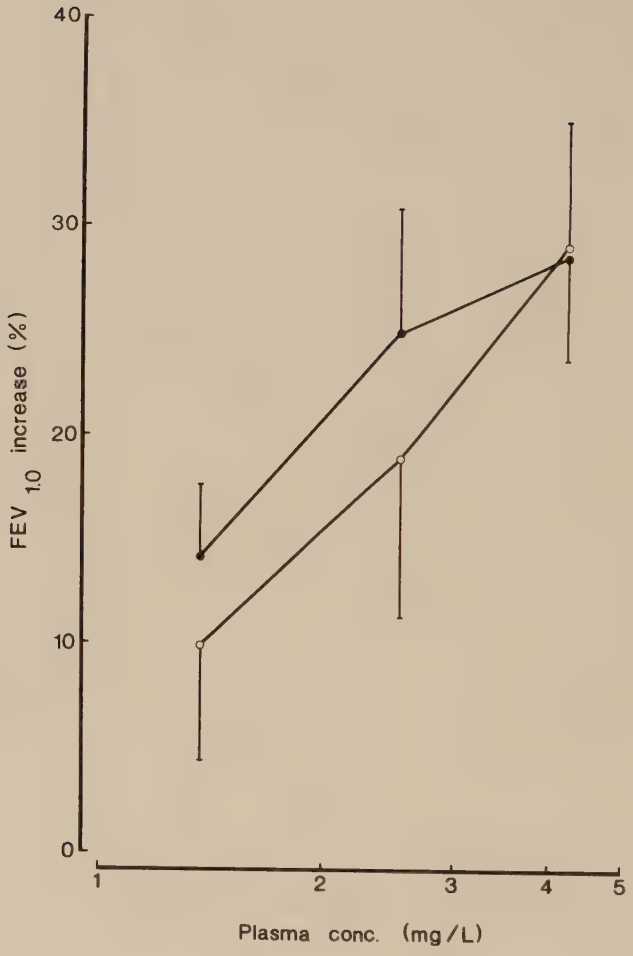

Fig. 4. Increase in FEV_1 versus plasma concentation of enprofylline fifty min after each infusion plotted semi-logarithmically. N = 8. Filled circles: Mean ± SEM of individual increases in FEV_1 over starting values. Open circles: Mean ± SEM of individual increases in FEV_1 over corresponding placebo values.
S.I. conversion: µmole/L = mg/L . 5.1

Circulation. Compared to placebo, enprofylline induced a significant rise in heart rate (Fig. 5). The difference was maximal, 13.0 $\pm$ 2.5 beats/min after 130 min, i.e., at the time for the peak concentration of enprofylline after the third infusion. Enprofylline lowered systolic and diastolic blood pressure maximally by 9.9 $\pm$ 5.7 and 6.0 $\pm$ 4.0 mm Hg, respectively. The changes were not statistically significant compared to the changes with placebo (Fig. 5).

Tremor. Tremor of hand was not affected by enprofylline or placebo (Fig. 5).

Side effects. No subjective side effects were recorded after enprofylline, nor after placebo.

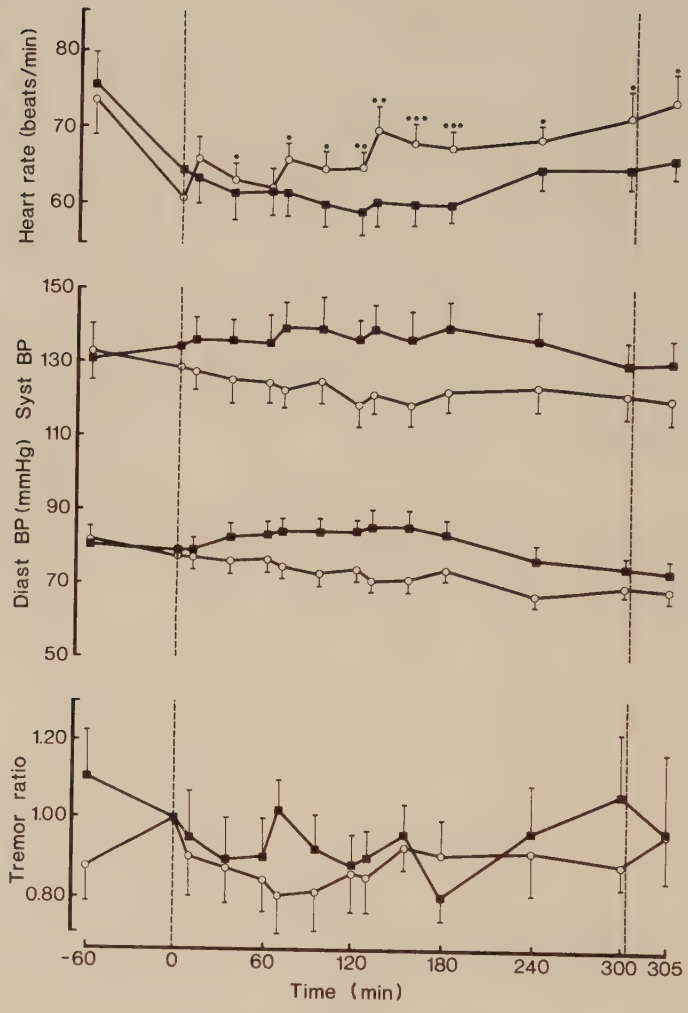

Fig. 5. Heart rate, systolic and diastolic blood pressure (BP) and tremor ratio after repeated 10-min infusions of 1 mg/kg of enprofylline and placebo in asthmatic patients. (Tremor ratio at time 0 is defined as 1.00, see 7, 15).
*** = p<0.001 ** = p<0.01 * = p<0.05

DISCUSSION

The present spirometric results show a marked and plasma concentration-related bronchodilating effect of enprofylline. The maximum effect on $FEV_{1.0}$ was similar to that recorded after inhalation of five doses of terbutaline aerosol at the end of the placebo day. The graded increments in $FEV_{1.0}$ corresponded well to the cumulated plasma concentrations. It cannot be excluded, however, that part of the effect after the third dose is due to a slowly developing effect of the previous doses . Still, the present data suggest that enprofylline produces concentration-dependent bronchodilation at least in the interval between 1 and 4 mg/L. This result may be compared with the recommended plasma levels of theophylline (5-20 mg/L) and agrees with earlier data suggesting that enprofylline is about five times more potent than theophylline as a bronchodilator (6, 7, 11, 13).

Xanthine pharmacology has obvious connections with many putative physiological roles of adenosine. It is now well established that clinically relevant concentrations of theophylline exert specific antagonism at cell surface receptor sites for adenosine (eg.3). Adenosine has potent actions in the CNS, the kidney, the cardiovascular system and other tissues, and it has been suggested that many effects of theophylline reflect adenosine receptor antagonism (eg.3, 14). In view of the widespread and increasing therapeutic usage of theophylline in asthma an understanding of the involvement of adenosine antagonism in the pulmonary effects of xanthines would be of particular interest. Different workers have suggested

that the antiasthmatic actions of these drugs could reflect adenosine receptor antagonism (2,3,14). The cumulating data obtained on enprofylline apparently are evidence against this view. In contrast to theophylline enprofylline has been shown to be without or almost without adenosine antagonistic potency in several systems representing different types of adenosine receptors; smooth muscle, cardiac and nervous tissues, thrombocytes, etc. (8,11,12 and unpublished work). These observations coupled with the potent lung actions of enprofylline (cf 6,8,13 and this study) do not support an important role for adenosine antagonism in the bronchodilator actions of xanthines. In this context adenosine antagonism would even be an undesirable characteristic (cf 8) due to the possible involvement of this mechanism in a number of theophyllines extrapulmonary effects.

Tremor was recorded in the present study becuase it would reflect a CNS-stimulant behavioural effect (cf 7,9). The patients participating had earlier been shown to respond to oral theophylline (plasma level 16.0 $\pm$ 0.8 mg/L) with significantly increased tremor (tremor ratio increased from 1.00 to 1.51). Oral enprofylline (3.0 $\pm$ 0.4 mg/L) was without any such action (7). The view that enprofylline does not induce hand tremor is supported by the present recordings obtained at three different plasma levels of the drug.

In accordance with previous experience (eg. 6), the cardiovascular effects of enprofylline were slight. However, a significant increase in heart rate was observed after enprofylline

in the present study. Both reflex and direct effects could have produced this increase since enprofylline has both vasodilating and positive chronotropic actions (cf 8, 11). It has been reported that theophylline given i.v. occasionally is associated with a large drop in blood pressure (1). The risk for this side effect is likely to remain with enprofylline.

In conclusion, enprofylline seems to be an antiasthmatic drug with promise. The results of the present study seem to make it particularly worthwhile to evaluate enprofylline for intravenous treatment of acute asthma.

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